the C.epsilon.4 domains. These loops are the. . . .near the interchain disulfide (328-336). The linker regions between the C.epsilon.2 and C.epsilon.3 domains are involved in interactions with the ***Fc*** .epsilon.RI.alpha., which cause both linker segments to point up and away from the complex interface. The role of the IgE- ***Fc*** C.epsilon.4 domains is to provide a structural dimerization scaffold that enables two C.epsilon.3 domains to form the bivalent interaction with ***Fc*** .epsilon.RIo.alpha..

DETD [0127] Biophysical studies of the IgE- ***Fc*** : ***Fc*** .alpha.FR.alpha. complex in solution indicate that a 1:1 complex is formed between the antibody and ***Fc*** .epsilon.RI.sup.20-23. This contrasts with models with a 2:1 stoichiometry that have been proposed for the interaction of the IgG antibody with the ***Fc*** .gamma.RIIa and ***Fc*** .gamma.RIIb receptors.sup.24-26, as well as with the crystal structure of the MHC-class I like neonatal ***Fc*** receptor with IgG.sup.27-29. The observation of a 1:1 complex in both of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex crystal forms is consistent with data on these complexes obtained using gel filtration and analytical ultracentrifugation techniques.sup.22,23. In principle, the 1:1 stoichiometry could arise due to ***Fc*** .epsilon.RI-induced conformational changes in the IgE- ***Fc*** , creating asymmetry in the ***Fc*** region, or by the binding of ***Fc*** .epsilon.RI across the ***Fc*** two-fold axis, creating a steric inhibition for the binding of a second receptor.

DETD [0128] FIGS. 2a and 2b show surface representations of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex, demonstrating how the convex surface of the receptor interacts asymmetrically with the two IgE- ***Fc*** C.epsilon.3 domains. The receptor is positioned near the ***Fc*** -diad axis. There are two structural keys that dictate the formation of complexes with this stoichiometry: (1) The induction of structural asymmetry in the IgE- ***Fc*** C.epsilon.2/C.epsilon.3 linker and (2) Steric hindrance that blocks the binding of a second receptor.

DETD [0129] Structural differences in the IgE- ***Fc*** domains are easily visualized by the superposition of the two C.epsilon.3 domains as shown in FIG. 2c. This superposition demonstrates that the C.epsilon.2/C.epsilon.3 linker regions comprised of residues 327-336, are constrained to an asymmetric arrangement by interactions with ***Fc*** .epsilon.RI. Other loops that are involved in distinct interactions with the two ***Fc*** .epsilon.RI binding sites also adopt slightly different conformations in the two C.epsilon.3 domains, such as the FG loops indicated in FIG.. . .

DETD . . . 2 creates a steric block of the binding of a second receptor. FIG. 2d shows representations of the both the IgE- ***Fc*** and ***Fc*** .epsilon.RI.alpha. in which the complex has been separated to exposed the buried interaction surfaces. The C.epsilon.2/C.epsilon.3 linker amino acids form the top of an arch that conforms to the convex surface of ***Fc*** .epsilon.RI.alpha., generating an asymmetric binding site for a single receptor. While some of the C.epsilon.3 binding surface remains accessible to the. . . a second receptor, superposition of a second receptor onto the 1:1 complex shows significant steric overlap between receptors and the IgE- ***Fc*** C.epsilon.2/C.epsilon.3 linker amino acids. Thus the binding of one receptor effectively prevents the binding of a second due to both the asymmetric arrangement of the IgE- ***Fc*** C.epsilon.2/C.epsilon.3 linker and by receptor binding across the ***Fc*** diad axis. Both contribute sterically to interfering with the binding of a second receptor. Although different residues in the ***Fc*** are used to form sites 1 and 2, there are four residues (R334, G335, V336, and H424) common to both sites, providing direct interactions that prevent the simultaneous binding of two receptors to one IgE- ***Fc*** .

DETD [0132] The receptor shows little change in conformation upon complex formation with the ***Fc*** . The overall RMS difference in 158 C.alpha. positions compared to the unbound receptor.sup.11 is 1.11 .ANG.. There are two loops on the receptor which adopt different conformations from those seen in the original ***Fc*** .epsilon.RI.alpha. structure.sup.11, the BC loop in D1 (residues 30-35) and the C' strand in D2 (residues 127-133). The D2 C' strand is longer in the ***Fc*** .epsilon.RI.alpha.:IgE- ***Fc*** complex compared to the ***Fc*** .epsilon.RI.alpha. structure alone. In the receptor structure, the C strand forms hydrogen bonds to the C' strand through

residue L127.sup.11, while in the complex, the main chain hydrogen bonds extend to Y 131. However, analysis of the ***Fc*** .epsilon.RI.alpha. structure in multiple crystal forms (Garman et al., in preparation) shows that the C' strand can adopt a variety of. . . BC loop in Domain 1 also adopts different conformations in different crystal forms, but this region is not involved in IgE- ***Fc*** interactions.

DETD [0133] The IgE- ***Fc*** in the complex is observed in a conformation that is very similar to the ***Fc*** domains of IgG antibodies.sup.30,31. Similar binding interactions between IgG antibodies and ***Fc*** .gamma.Rs could form an analogous 1:1 complex, as suggested by biophysical studies of the IgG- ***Fc*** interaction with ***Fc*** .gamma.RIII.sup.32. In contrast to the similarities of the bound IgE- ***Fc*** to IgG- ***Fc*** structures, the crystal structure of the IgE- ***Fc*** alone shows a large re-arrangement of the two C.epsilon.3 domains that is greater than the conformational variation observed in IgG- ***Fc*** structures (see P_AL-9, ibid.). The IgE- ***Fc*** conformation may change substantially from the unbound conformation, which may exist in multiple conformational states that interact weakly with the receptor. This conformational variation in the IgE- ***Fc*** structure suggests new avenues to inhibiting IgE-receptor interactions using allosteric modulators that could stabilize the closed, unbound IgE- ***Fc*** structure.

DETD [0134] F. Details of the Binding Surfaces of the ***FcR*** :IgE Interaction

DETD [0135] The surface areas of both the IgE- ***Fc*** and ***Fc*** .epsilon.RI.alpha. that are involved in binding are shown in FIG. 2d, forming a total buried surface of .about.1890 .ANG..sup.2. The IgE- ***Fc*** adopts a concave or crown-like configuration at the N-terminal ends of the two C.epsilon.3 domains that matches the convex shape. . . receptor that involve an overlapping but non-identical set of IgE residues in each of these two sites. Of the fifteen ***Fc*** .epsilon.RI residues that contact the IgE- ***Fc*** , seven are aromatic and five of these aromatic residues are surface exposed tryptophans. In contrast, of the nineteen IgE- ***Fc*** residues that contact the ***Fc*** .epsilon.RI.alpha., none are aromatic. The large fraction of aromatic receptor residues that are involved in this interaction and the large buried. . .

DETD [0136] FIG. 3a shows a plot of the IgE- ***Fc*** residues that are buried in the interaction with the receptor. C.epsilon.3 residues involved in Site 1 are in the top half of the plot and form specific interactions with ***Fc*** .epsilon.RI.alpha. residues shown in FIG. 3b. Nine amino acids from the IgE and seven amino acids from the receptor form Site. . . 4a), burying a total of .about.835 .ANG..sup.2 of surface area. The IgE residues are from four distinct regions of the IgE- ***Fc*** sequence that are predominantly loop and adjacent strand residues, including the N-terminal linker (residues 334-336), the BC loop (residues 362-364),. . .

DETD . . . regions of the sequence, including extensive interactions with the C.epsilon.2/C.epsilon.3-linker region (residues 332-336) and the FG loop (residues 424-427). The ***Fc*** .epsilon.RI.alpha. residues are from three regions of the sequence (FIGS. 3c and 4b), the D1D2 linker region (residues 85-87), the BC. . . 113) and the FG loop (residues 156-158). Residues from the receptor D1 domain do not form direct interactions with the IgE- ***Fc*** , but are likely important for stabilizing the conformation of the D1D2 linker residues, including the highly conserved W87 (FIGS. 3c. . .

DETD [0139] One of these molecules sits above ***Fc*** .epsilon.RI-W156 and below the C.epsilon.3-FG loop near H424 in Site 2 (FIG. 3d). The position of the CHAPS heterocyclic core is analogous to the position of the ***FcR*** C' loop residues in Site 1. Although the CHAPS interaction may be weak, this structure provides a foundation for using. . . be a viable inhibitor of the IgE binding, given mutagenesis data that indicate the importance of this site in overall IgE: ***Fc*** .epsilon.RI affinity. In addition, H424, which is located next to the CHAPS binding site, makes contacts with the receptor in both. . .

DETD [0140] H. Locations of IgE and ***FcR*** Mutations in the Structure of the Complex.

DETD [0141] Mutagenesis studies of both the IgE- ***Fc*** and ***Fc*** .epsilon.RI.alpha. have been carried out in efforts to define the residues in both proteins that contribute to the stability of the

complex. For ***Fc*** .epsilon.RI.alpha., these studies have implicated residues located in the D2 domain, including amino acids 87, 113, 115, 117, 118, 120, 121,. . . location of these residues is consistent with the observed complex, not all of the residues make direct contacts with the IgE- ***Fc*** , as shown in FIGS. 4a and 4b. Of the residues identified by mutagenesis techniques, eight are observed to interact directly. . .

DETD [0142] The identification of the IgE- ***Fc*** binding site for receptor has implicated regions near the C.epsilon.2/C.epsilon.3 linker, the C.epsilon.3-AB helix and the C.epsilon.3-CD loop.sup.12,15,16,40,41. In general, most studies concur that the C.epsilon.2 and C.epsilon.4 domains do not interact directly with antibody. Residues in the IgE- ***Fc*** AB helix are likely to have an indirect effect on receptor binding, by altering the flexibility and geometry of the. . . techniques have identified residues 333, 334, 376, 378, 380, 393, 414, 427 and 430 as possible contact residues in the IgE- ***Fc*** . Of these residues, three are observed as contact residues (333, 334, 427), one is within three residues (430) of a contact. However, four of these residues are located in the CD loop of C.epsilon.3 and are distant from the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. interface (376, 378, 380, 414). Not all mutations at these residues are deleterious, for example R376A or R376K has little. . .

DETD . . . largest number of atomic contacts across the respective interfaces. Also shown are the residues that are found in the related ***human*** IgG receptors (***Fc*** .gamma.R1, ***Fc*** .gamma.RII and ***Fc*** .gamma.RIII, to the left) and in four subtypes of IgG antibodies (to the right).

DETD [0145] In Site 1 there is little conservation of the residues that form the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. interface. Three residues are completely conserved (IgE residues 335, 362 and 394) in the ***Fc*** sequences, while there is poor conservation in the receptor sequences, except for the partial conservation of K117 and the relatively. . . substitution of Y129 for F or Y in the IgG receptors also suggests that this site may be found in IgG- ***Fc*** complexes with the ***Fc*** .gamma.Rs. However, Y131, which forms a large number of atomic contacts across the interface and is buried in a shallow surface pocket on the IgE- ***Fc*** , is not conserved in the ***Fc*** .gamma.Rs (changing to either H or R). Given the central location of Y131 to the IgE interface, this residue may play. . . specificity (FIG. 3b). For example, four of the five contact residues in IgE for Y131 are also different in the IgG- ***Fc*** sequences. In general, residues within the four IgG subtypes are highly conserved in the Site 1 interface (7/9 identical), as compared to the significant variation in the ***Fc*** .gamma.R residues. FIG. 4b shows the conservation of interactions that are central to the Site 2 interface. P426 and L425 are absolutely conserved in all IgG ***Fc*** sequences and P426 interacts with two absolutely conserved tryptophans in the ***Fc*** .epsilon.R complex (W87 and W110). The two tryptophans form a hydrophobic pocket on the surface of the receptor into which the. . . Site 2 also includes three residues (IgE residues 332-334) that have been shown to affect binding of IgG subtypes to ***Fc*** .gamma.RI. IgG1 binds with high affinity to ***Fc*** .gamma.RI, whereas IgG2 does not, and the difference in binding affinity can be introduced into IgG1 by the substitution of residues LLG to PVA (IgE residues 332-334, highlighted in black in FIG. 4b).sup.42,.sup.43. This region of the IgE- ***Fc*** interacts with the ***Fc*** .epsilon.RI.alpha. FG loop residues 156-158 (FIGS. 3c and 4b). Previous mutagenesis experiments have also shown that the transfer of the ***Fc*** .epsilon.RI.alpha. FG loop to ***Fc*** .gamma.RII confers detectable IgE binding.sup.44. Thus, residues involved in the formation of Site 2 are implicated in the binding and specificity of both IgE and IgG ***FcRs*** , consistent with a conserved binding mode across these members of the ***FcR*** family. Overall, five residues are completely conserved in these ***human*** receptors and IgG sequences that could form a common set of contacts. Variation in the ***Fc*** .gamma.R FG loop sequences that contact the N-terminal linker region of the ***Fc*** fragment may provide key interactions that modulate the affinity of interaction of specific ***FcR*** :IgG pairs.

DETD [0147] The crystal structure of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex clarifies the atomic interactions that regulate the specificity and stoichiometry of protein:protein

interactions underlying allergy and anaphylaxis. Similar complexes. . . suggested by previous mutagenesis studies and the structural analysis presented here, in contrast to models proposed for the interaction of IgG- ***Fc*** with the low affinity receptor, ***Fc*** .gamma.RIIb.sup.25 and ***Fc*** .gamma.RIIa.sup.24. Knowledge of these interactions may allow the development of inhibitors for the treatments of allergy and asthma and may also facilitate the targeted engineering of therapeutic antibodies to interact with specific subsets of the ***FcR*** family.sup.45.

DETD . . . scheme for IgE binding shown in FIG. 5. In this scheme, the independent binding of each C.epsilon.3 domain in the ***Fc*** .epsilon.RI.alpha. complex, leads to two pathways for the full dissociation of the complex. Surface plasmon resonance studies of IgE- ***Fc*** dissociation show two distinct kinetic dissociation rates that were hypothesized to represent the interaction of two different binding interactions between the IgE- ***Fc*** and ***Fc*** .epsilon.RI.alpha., consistent with this kinetic scheme.sup.16,41. The IgE- ***Fc*** mutation R334S affects the biphasic dissociation kinetics of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex by selectively accelerating the slow dissociation rate.sup.16. R334 is used in distinct and specific ways in Site 1 and. . .

DETD [0149] A model for the formation of a complex between an intact IgG antibody and ***Fc*** -receptor is shown in FIG. 6. In this model the crystal structure of the low affinity IgG receptor (***Fc*** .gamma.RIIb).sup.25 and one of the available intact IgG antibody structures (1IGY).sup.46 were superimposed on the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. complex. Superposition of the IgG structure is based on the Site 2 interactions, and this places the second IgG- ***Fc*** Cg2 domain within close proximity of the Site 1 binding surface without any conformational rearrangements (FIG. 6). The Fab arms of IgG are flexible and are also easily accommodated into this complex. Antigen-induced crosslinking of antibody: ***FcR*** complexes, leads to the co-localization of ***Fc*** receptors and the initiation of intracellular signal transduction cascades.sup.2,47. Within the one of the IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. crystal forms and the IgE- ***Fc*** crystals (60/189,403, ibid.), C.epsilon.3 domains from adjacent molecules are observed to form packing interactions in the crystal through a strand. . .

DETD [0151] 1. Crystallization of the ***Human*** IgE- ***Fc*** : ***Fc*** .epsilon.RI.alpha. Complex

DETD [0152] ***Human*** IgE- ***Fc*** C.epsilon.3/C.epsilon.4 domains and a carbohydrate mutant of the ***Fc*** .epsilon.RI.alpha..sup.11 were expressed in insect cells essentially as described for IgE- ***Fc*** C.epsilon.3/C.epsilon.4 in 60/189,403. Complexes of wt- ***Fc*** -C.epsilon.3/C.epsilon.4 and wt- ***Fc*** .epsilon.RI.alpha. produced only poorly diffracting crystals. Since the receptor is heavily glycosylated (.about.33% carbohydrate by weight), and the carbohydrate sites are. . . sites (residues 74, 135, and 140) located on both D1 and D2 in the receptor structure. The triple receptor mutant, ***Fc*** .epsilon.RI.alpha..DELTA.4-6 was subcloned into the pvl1392 baculovirus transfer vector and recombinant virus produced. The mutant receptor was active, expressed well and was purified by affinity chromatography similarly to the wt protein. Purified wt- ***Fc*** and (.alpha..DELTA.4-6 or wt-.alpha. were incubated to form complex, which was subsequently purified by gel filtration chromatography using a Pharmacia. . . concentrated to 10 mgs/ml. Crystallizations were carried out using the the hanging drop method of vapor diffusion. Crystals of the wtIgE- ***Fc*** :wt- ***Fc*** .epsilon.RI.alpha. complex were grown from 1.4-1.6M Ammonium Sulfate, 100 mM Tris pH 8.5, over a period of 8-12 months (Form I). Purified wtIgE- ***Fc*** :.alpha.A4-6 complex was crystallized using 100 mM Tris, pH 8.5, 1.4-1.6M Ammonium Sulfate, and 8 mM CHAPS at room temperature. Crystals. . .

DETD . . . AMoRe was critical to the success of the search. Both AMoRe and EPMR produced crystallographically equivalent locations for the receptor. 2Fo- ***Fc*** electron density maps with phases from the receptor revealed density corresponding to the two C.epsilon.3 portions of the ***Fc*** . A model for the core residues of C.epsilon.3 was created (see 60/189,403, ibid.) based upon homologous residues from an intact IgG structure 1IGT 46 A new 2Fo- ***Fc*** map was created with phases from the receptor and core residues of C.epsilon.3. This map

showed density for the locations. . . refinement of the receptor, the core residues in C.epsilon.3, and the core residues in C.epsilon.4 reduced the Rfree to 45%. 2Fo- ***Fc*** maps and composite omit maps revealed clear density for protein and carbohydrate atoms absent from the model. The Form I. . .

DETD . . . Form II data using the CNS program.sup.19. Non-crystallographic symmetry restraints of 300 kcal/mol/A.sup.2 were imposed on all atoms in the ***Fc*** except the loops that interact with the receptor. Refinement was performed using all data from 40-3.25 .ANG. with |F|>0 and. . . inserting all the missing loops from the protein chains, CHAPS molecules were located as large peaks of positive density in Fo- ***Fc*** maps. The current refinement statistics are summarized in

TABLE 3

Figures were made using the programs Molscript.sup.51 and Grasp.sup.52.
Data Collection and. . . II, 3.25 .ANG.)

Rfactor/Rfree	Total # atoms	Protein	Carbohydrate	Detergent	Sulfate
25.8/28.1	5251	4821	259	146	25

RMSD Bonds	Angles	Average B Overall	Receptor	***Fc*** chain 1	***Fc*** chain 2
0.0102	1.58	91.0	63.2	94.9	99.4

Ramachandran	Favored	Allowed	Generous	Disallowed
	77.0%	21.5%	1.5%	0.0%

.dagger-dbl.Last shell is shown in parentheses. . .

CLM What is claimed is:

. . . selected from the group consisting of: (a) a three-dimensional model of a complex between (i) an extracellular domain of a ***human*** high affinity ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .epsilon.RI.alpha.) protein and (ii) a ***human*** IgE ***Fc*** region comprising C.epsilon.3 and C.epsilon.4 domains (***Fc*** -C.epsilon.3/C.epsilon.4), wherein said model substantially represents the atomic coordinates specified in Table 1; and (b) a three-dimensional model comprising a modification of said model of (a), wherein said modification represents a complex between a ***Fc*** receptor protein that binds to a ***Fc*** domain of an antibody and an antibody ***Fc*** region that binds to a ***Fc*** receptor protein the hinge between domain C.epsilon.3 and domain C.epsilon.4 of the ***Fc*** -C.epsilon.3/C.epsilon.4 region, and a ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 region that interacts with 3-[3-(cholamidopropyl) dimethylammonio]-1-propane-sulfonate (CHAPS).

. . . produce the three-dimensional model of claim 1, wherein the three-dimensional model is the complex between the extracellular domain of a ***human*** ***Fc*** .epsilon.RI.alpha. protein and a ***human*** ***Fc*** -C.epsilon.3/C.epsilon.4 region, said method comprising representing amino acids of said protein and said region in said complex at substantially the atomic. . .

. . . the three-dimensional model of a complex between (i) an extracellular antibody binding domain of an antibody receptor protein other than ***human*** ***Fc*** .epsilon.RI.alpha. as represented by coordinates in Table 1 and (ii) an antibody receptor binding domain of an antibody other than ***human*** IgE as represented by coordinates in Table 1, said method comprising homology modeling.

4. An isolated crystal of a complex between an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein and an IgE ***Fc*** -C.epsilon.3/C.epsilon.4 region.

. . . A method to produce the isolated crystal of claim 4, wherein the

complex is between an extracellular domain of a ***Fc*** .epsilon.RI.alpha. protein and an IgE ***Fc*** -C.epsilon.3/C.epsilon.4 region, said method comprising vapor diffusion.

6. A method to identify a compound that inhibits the binding between an IgE antibody and a ***Fc*** .epsilon.RI.alpha. protein, said method comprising using a three-dimensional model of a complex between an extracellular domain of a ***human*** high affinity ***Fc*** .epsilon.RI.alpha. protein and a ***human*** ***Fc*** -C.epsilon.3/C.epsilon.4 region to identify said compound, wherein said model substantially represents the atomic coordinates specified in Table 1.

10. A compound that inhibits the binding between an IgE antibody and a ***Fc*** .epsilon.RI.alpha. protein, said compound identified by analysis of a three-dimensional model of a complex between an extracellular domain of a ***human*** high affinity ***Fc*** .epsilon.RI.alpha. protein and a ***human*** ***Fc*** -C.epsilon.3/C.epsilon.4 region to identify said compound, wherein said model substantially represents the atomic coordinates specified in Table 1.

11. A polypeptide selected from the group consisting of a ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 1, a ***Fc*** .epsilon.RI.alpha.: ***Fc*** -C.epsilon.3/C.epsilon.4 interaction site 2, a C strand of domain 2 of ***Fc*** .epsilon.RI.alpha., a C'E loop of domain 2 of ***Fc*** .epsilon.RI.alpha., a tryptophan-containing hydrophobic ridge of ***Fc*** .epsilon.RI.alpha., a crystal contact cluster involved in IgE binding; a FG loop in D2; a D1D2 interface; a cleft between D1. . .
14. The method of claim 13, wherein said target protein is a ***Fc*** -C.epsilon.3/C.epsilon.4 protein, a ***Fc*** .epsilon.RI.alpha. protein or a protein comprising SEQ.ID NO.2, and wherein said improved function is selected from the group comprising: (a) increased stability, increased affinity for an IgE binding domain of a ***Fc*** .epsilon.RI.alpha. protein, altered substrate specificity or increased solubility when said target protein is the ***Fc*** -C.epsilon.3/C.epsilon.4 protein; and (b) increased stability, increased affinity for an ***Fc*** -domain of an antibody, altered substrate specificity or increased stability when said target protein is the ***Fc*** .epsilon.RI.alpha. protein or the protein comprising SEQ.ID NO.2.

. . . wherein said mutein has at least one improved function compared to the target protein, wherein said target protein is the ***Fc*** .epsilon.RI.alpha. protein, the ***Fc*** -C.epsilon.3/C.epsilon.4 protein or the protein comprising SEQ.ID NO.2.

16. The mutein of claim 15 having an improved function compared to an unmodified ***Fc*** .epsilon.RI.alpha. protein, wherein the amino acid sequence of said mutein differs in at least one position from the amino acid sequence. . .

L13 ANSWER 10 OF 24 USPATFULL on STN
AN 2003:168820 USPATFULL
TI Equine ***Fc*** ***epsilon*** ***receptor*** alpha chain proteins and uses thereof.
IN Weber, Eric R., Fort Collins, CO, United States
McCall, Catherine A., Boulder, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6582701 B1 20030624
AI US 2000-515311 20000229 (9)
RLI Division of Ser. No. US 1998-15734, filed on 29 Jan 1998, now patented, Pat. No. US 6057127
DT Utility
FS GRANTED
EXNAM Primary Examiner: Chan, Christina; Assistant Examiner: Huynh, Phuong N.
LREP Heska Corporation
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 0 Drawing Figure(s); 0 Drawing Page(s)

LN.CNT 2628
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to equine ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect IgE using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

TI Equine ***Fc*** ***epsilon*** ***receptor*** alpha chain proteins and uses thereof

AB The present invention relates to equine ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of. . . such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

PARN . . . a Divisional Application of application Ser. No. 09/015,734, filed Jan. 29, 1998, now U.S. Pat. No. 6,057,127 entitled "NOVEL EQUINE ***Fc*** ***EPSILON*** ***RECEPTOR*** ALPHA CHAIN NUCLEIC ACID MOLECULES, PROTEINS AND USES THEREOF", which is incorporated herein by this reference in its entirety.

SUMM The present invention relates to equine ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of. . .

SUMM Immunological stimulation can be mediated by IgE antibodies when IgE complexes with ***Fc*** ***epsilon*** ***receptors*** . ***Fc*** ***epsilon*** ***receptors*** are found on the surface of certain cell types, such as mast cells. Mast cells store biological mediators including histamine, prostaglandins and proteases. Release of these biological mediators is triggered when IgE antibodies complex with ***Fc*** ***epsilon*** ***receptors*** on the surface of a cell. Clinical symptoms result from the release of the biological mediators into the tissue of. . .

SUMM The discovery of the present invention includes a novel equine ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon.R) alpha chain protein and the use of such a protein to detect the presence of IgE in a putative IgE-containing composition; to identify inhibitors of biological responses mediated by an equine ***Fc*** .sub..epsilon.R protein; and as a therapeutic compound to prevent or treat clinical symptoms that result from equine ***Fc*** .sub..epsilon.R-mediated biological responses.

SUMM Prior investigators have disclosed the nucleic acid sequence for: the ***human*** ***Fc*** .sub..epsilon.R alpha chain (Kochan et al., Nucleic Acids Res. 16:3584, 1988; Shimizu et al., Proc. Natl. Acad. Sci. USA 85:1907-1911, 1988; and Pang et al., J. Immunol. 151:6166-6174, 1993); the ***human*** ***Fc*** .sub..epsilon.R beta chain (Kuster et al., J. Biol. Chem. 267:12782-12787, 1992); the ***human*** ***Fc*** .sub..epsilon.R gamma chain (Kuster et al., J. Biol. Chem. 265:6448-6452, 1990); and the canine ***Fc*** .sub..epsilon.R alpha chain (GenBank.TM. accession number D16413). Although the subunits of ***human*** ***Fc*** .sub..epsilon.R have been known as early as 1988, they have never been used to identify an equine ***Fc*** .sub..epsilon.R. Similarly, even though the canine ***Fc*** .sub..epsilon.R chain has been known since 1993, it has never been used to identify an equine ***Fc*** .sub..epsilon.R. Moreover, the determination of ***human*** and canine ***Fc*** ***epsilon*** ***receptor*** sequences does not indicate, suggest or predict the cloning of a novel ***Fc*** .sub..epsilon.R gene from a different species, in particular, from an equine species. Previous investigators have found a low degree of similarity between rat, mouse and ***human*** ***Fc*** .sub..epsilon.R.alpha. (Ravtech et al., Ann. Rev. Immunol. Vol. 9, pp. 457-492, 1991). Thus, given this low degree of sequence similarity, it would appear only "obvious to try" to obtain an equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecule and protein.

SUMM . . . invention are needed in the art that will provide specific detection of IgE, in particular equine IgE, and treatment of ***Fc*** ***epsilon*** ***receptor*** -mediated disease.

SUMM The present invention relates to a novel product and process for detecting IgE and protecting animals from ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. According to the present invention there are provided equine ***Fc*** .sub..epsilon.R proteins and mimetopes thereof; equine ***Fc*** .sub..epsilon.R nucleic acid molecules, including those that encode such proteins; antibodies raised against such equine ***Fc*** .sub..epsilon.R proteins (i.e., anti-equine ***Fc*** .sub..epsilon.R antibodies); and other compounds that inhibit the ability of equine ***Fc*** .sub..epsilon.R protein to form a complex with IgE (i.e, inhibitory compounds or inhibitors).

SUMM . . . comprising such proteins, mimetopes, nucleic acid molecules, antibodies, and/or inhibitory compounds, as well as use of such therapeutic compositions to ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

SUMM One embodiment of the present invention is an isolated nucleic acid molecule encoding an equine ***Fc*** .sub..epsilon.R protein. The equine ***Fc*** .sub..epsilon.R protein preferably includes: proteins comprising amino acid sequences SEQ ID NO:2, SEQ ID NO:7 and SEQ ID NO:12; and proteins. . . by allelic variants of nucleic acid molecules encoding a protein comprising any of the amino acid sequences. Particularly preferred equine ***Fc*** .sub..epsilon.R nucleic acid molecules include: nucleic acid molecules comprising nucleic acid sequences SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4,. . .

SUMM The present invention also includes an isolated equine ***Fc*** .sub..epsilon.R protein. A preferred equine ***Fc*** .sub..epsilon.R protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a nucleic acid sequence including SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:8. Particularly preferred equine ***Fc*** .sub..epsilon.R proteins include at least one of the following amino acid sequences: SEQ ID NO:2, SEQ ID NO:7 and SEQ ID. . .

SUMM The present invention also relates to recombinant molecules, recombinant viruses and recombinant cells that include equine ***Fc*** .sub..epsilon.R nucleic acid molecules of the present invention. Also included are methods to produce such nucleic acid molecules, recombinant molecules, recombinant. . .

SUMM . . . detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated equine ***Fc*** .sub..epsilon.R molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon.R molecule:IgE complex; and (b) determining the presence of IgE by detecting the ***Fc*** .sub..epsilon.R molecule:IgE complex, the presence of the ***FC*** .sub..epsilon.R molecule:IgE complex indicating the presence of IgE. A preferred equine ***Fc*** .sub..epsilon.R molecule is one in which a carbohydrate group of the equine ***Fc*** .sub..epsilon.R molecule is conjugated to biotin.

SUMM . . . IgE-containing composition under conditions suitable for formation of a recombinant cell:IgE complex, in which the recombinant cell comprises an equine ***Fc*** .sub..epsilon.R molecule; and (b) determining the presence of IgE by detecting the recombinant cell:IgE complex, the presence of the recombinant cell:IgE complex indicating the presence of IgE. A preferred method to detect IgE comprises: (a) immobilizing the ***Fc*** .sub..epsilon.R molecule on a substrate; (b) contacting the ***Fc*** .sub..epsilon.R molecule with the putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon.R molecule:IgE complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain ***Fc*** .sub..epsilon.R molecule:IgE complex binding to the substrate; and (d) detecting the presence of the ***Fc*** .sub..epsilon.R molecule:IgE complex. Another preferred method to detect IgE comprises: (a) immobilizing a specific antigen on a substrate; (b) contacting the. . . binding to said substrate; and (d) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with said ***Fc*** .sub..epsilon.R molecule. Another preferred method to detect IgE comprises: (a) immobilizing an antibody that binds selectively to IgE on a substrate;. . . binding to the substrate; and (d) detecting the presence of the antibody:IgE complex by contacting the antibody:IgE complex with said ***Fc*** .sub..epsilon.R molecule. Another preferred method to detect IgE

comprises: (a) immobilizing a putative IgE-containing composition on a substrate; (b) contacting the composition with the ***Fc*** .sub..epsilon.R molecule under conditions suitable for formation of a ***Fc*** .sub..epsilon.R molecule:IgE complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain ***Fc*** .sub..epsilon.R molecule:IgE complex binding to the substrate; and (d) detecting the presence of the ***Fc*** .sub..epsilon.R molecule:IgE complex.

SUMM . . . a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising an equine ***Fc*** .sub..epsilon.R protein and a means for detecting IgE.

SUMM The present invention also includes an inhibitor that interferes with formation of a complex between equine ***Fc*** .sub..epsilon.R protein and IgE, in which the inhibitor is identified by its ability to interfere with the complex formation. A particularly preferred inhibitor includes a substrate analog of an equine ***Fc*** .sub..epsilon.R protein, a mimetope of an equine ***Fc*** .sub..epsilon.R protein and a soluble portion of an equine ***Fc*** .sub..epsilon.R protein. Also included is a method to identify a compound that interferes with formation of a complex between equine ***Fc*** .sub..epsilon.R protein and IgE, the method comprising: (a) contacting an isolated equine ***Fc*** .sub..epsilon.R protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the equine ***Fc*** .sub..epsilon.R protein forms a complex with IgE; and (b) determining if the putative inhibitory compound inhibits the complex formation. A test kit is also included to identify a compound capable of interfering with formation of a complex between an equine ***Fc*** .sub..epsilon.R protein and IgE, the test kit comprising an isolated equine ***Fc*** .sub..epsilon.R protein that can complex with IgE and a means for determining the extent of interference of the complex formation in. . . .

SUMM Yet another embodiment of the present invention is a therapeutic composition that is capable of reducing ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. Such a therapeutic composition includes one or more of the following therapeutic compounds: an isolated equine ***Fc*** .sub..epsilon.R protein; a mimetope of an equine ***Fc*** .sub..epsilon.R protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with an equine ***Fc*** .sub..epsilon.R gene; an isolated antibody that selectively binds to an equine ***Fc*** .sub..epsilon.R protein; and an inhibitor that interferes with formation of a complex between an equine ***Fc*** .sub..epsilon.R protein and IgE. A method of the present invention includes the step of administering to an animal a therapeutic composition. . . .

SUMM Yet another embodiment of the present invention is a method to produce an equine ***Fc*** .sub..epsilon.R protein, the method comprising culturing a cell transformed with a nucleic acid molecule encoding an equine ***Fc*** .sub..epsilon.R protein.

SUMM The present invention provides for isolated equine ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .sub..epsilon.R.alpha.) proteins, isolated equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules, antibodies directed against equine ***Fc*** .sub..epsilon.R.alpha. proteins and other inhibitors of equine ***Fc*** .sub..epsilon.R.alpha. activity. As used herein, the terms isolated equine ***Fc*** .sub..epsilon.R.alpha. proteins and isolated equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules refers to ***Fc*** .sub..epsilon.R.alpha. proteins and ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules derived from horses and, as such, can be obtained from their natural source or can be produced. . . of the present invention are advantageous because they enable the detection of IgE and the inhibition of IgE or equine ***Fc*** .sub..epsilon.R.alpha. protein activity associated with disease. As used herein, equine ***Fc*** epsilon alpha chain receptor protein can be referred to as ***Fc*** .sub..epsilon.R.alpha. protein or ***Fc*** .sub..epsilon.R alpha chain protein.

SUMM One embodiment of the present invention is an isolated protein comprising an equine ***Fc*** .sub..epsilon.R.alpha. protein. It is to be noted that the term "a" or "an" entity refers to one or more of that. . . .

SUMM As used herein, an isolated equine ***Fc*** .sub..epsilon.R.alpha.

protein can be a full length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. Preferably, an equine ***Fc*** .sub..epsilon.R.alpha. protein comprises at least a portion of an equine ***Fc*** .sub..epsilon.R.alpha. protein that binds to IgE, i.e., that is capable of forming a complex with an IgE.

SUMM An equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to bind to IgE. Examples of equine ***Fc*** .sub..epsilon.R.alpha. protein homologs include equine ***Fc*** .sub..epsilon.R.alpha. proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted,. . .

SUMM Equine ***Fc*** .sub..epsilon.R.alpha. protein homologs can be the result of natural allelic variation or natural mutation. Equine ***Fc*** .sub..epsilon.R.alpha. protein homologs of the present invention can also be produced using techniques known in the art including, but not limited. . .

SUMM Isolated equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention have the further characteristic of being encoded by nucleic acid molecules that hybridize under stringent hybridization conditions to a gene encoding an equine ***Fc*** .sub..epsilon.R.alpha. protein. As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules, including oligonucleotides, are. . .

SUMM As used herein, an equine ***Fc*** .sub..epsilon.R.alpha. gene includes all nucleic acid sequences related to a natural equine ***Fc*** .sub..epsilon.R.alpha. gene such as regulatory regions that control production of the equine ***Fc*** .sub..epsilon.R.alpha. protein encoded by that gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. In one embodiment, an equine ***Fc*** .sub..epsilon.R.alpha. gene of the present invention includes nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID. . .

SUMM . . . other nucleic acid and protein sequences presented herein) represent apparent nucleic acid sequences of certain nucleic acid molecules encoding equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention.

SUMM In another embodiment, an equine ***Fc*** .sub..epsilon.R.alpha. gene can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:1, SEQ ID. . . NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8 and/or SEQ ID NO:11. An allelic variant of an equine ***Fc*** .sub..epsilon.R.alpha. gene is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including. . .

SUMM The minimal size of a ***Fc*** .sub..epsilon.R.alpha. protein homolog of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of. . . length if they are AT-rich. As such, the minimal size of a nucleic acid molecule used to encode an equine ***Fc*** .sub..epsilon.R.alpha. protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of an equine ***Fc*** .sub..epsilon.R.alpha. protein homolog of the present invention is from about 4 to about 6 amino acids in length. There is no. . .

SUMM . . . used herein, an equine refers to any member of the horse family. Examples of horses from which to isolate equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention (including isolation of the natural protein or production of the protein by recombinant or synthetic. . .

SUMM Suitable horse cells from which to isolate an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention include cells that have ***Fc*** .sub..epsilon.R.alpha. proteins. Preferred horse cells from which to obtain an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention include basophil cells, mast cells, mastocytoma cells, dendritic cells, B lymphocytes, macrophages, eosinophils, and/or monocytes. An equine ***Fc*** .sub..epsilon.R.alpha. of the present invention is preferably obtained from mastocytoma cells, mast cells or basophil cells.

SUMM The present invention also includes mimetopes of equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention. As used herein, a mimetope of an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention refers to any compound that is able to

mimic the activity of such an equine ***Fc*** .sub..epsilon.R.alpha. protein (e.g., ability to bind to IgE), often because the mimetope has a structure that mimics the equine ***Fc*** .sub..epsilon.R.alpha. protein. It is to be noted, however, that the mimetope need not have a structure similar to an equine ***Fc*** .sub..epsilon.R.alpha. protein as long as the mimetope functionally mimics the protein. Mimetopes can be, but are not limited to: peptides that. . . nucleic acids; and/or any other peptidomimetic compounds. Mimetopes of the present invention can be designed using computer-generated structures of equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides. . . other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., an equine IgE ***Fc*** domain or anti-equine ***Fc*** .sub..epsilon.R.alpha. antibody). A mimetope can also be obtained by, for example, rational drug design. In a rational drug design procedure, the. . . for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of equine ***Fc*** .sub..epsilon.R.alpha. mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex.TM. technology, peptides identified by random screening of peptide libraries and proteins identified. . . by phage display technology. A preferred mimetope is a peptidomimetic compound that is structurally and/or functionally similar to an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention, particularly to the IgE ***Fc*** domain binding site of the equine ***Fc*** .sub..epsilon.R.alpha. protein. As used herein, the ***Fc*** domain of an antibody refers to the portion of an immunoglobulin that has ***Fc*** receptor binding effector function. Typically, the ***Fc*** domain of an IgE comprises the CH2 and CH3 domains of the heavy chain constant region.

SUMM According to the present invention, an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention refers to: an equine ***Fc*** .sub..epsilon.R.alpha. protein, in particular a soluble equine ***Fc*** .sub..epsilon.R.alpha. protein; an equine ***Fc*** .sub..epsilon.R.alpha. homolog; an equine ***Fc*** .sub..epsilon.R.alpha. mimetope; an equine ***Fc*** .sub..epsilon.R.alpha. substrate analog; or an equine ***Fc*** .sub..epsilon.R.alpha. peptide. Preferably, an equine ***Fc*** .sub..epsilon.R.alpha. molecule binds to IgE.

SUMM One embodiment of an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention is a fusion protein that includes an equine ***Fc*** .sub..epsilon.R.alpha. protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but. . . to, segments that can: enhance a protein's stability; act as an immunopotentiator to enhance an immune response against an equine ***Fc*** .sub..epsilon.R.alpha. protein; and/or assist purification of an equine ***Fc*** .sub..epsilon.R.alpha. protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired. . . protein, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the equine ***Fc*** .sub..epsilon.R.alpha.-containing domain of the protein and can be susceptible to cleavage in order to enable straight-forward recovery of an equine ***Fc*** .sub..epsilon.R.alpha. protein. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid molecule that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of an equine ***Fc*** .sub..epsilon.R.alpha.-containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; ***Fc*** receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); a "tag" domain (e.g., at. . . the domain, such as monoclonal antibodies); and/or a linker and enzyme domain (e.g., alkaline phosphatase domain connected to an equine ***Fc*** .sub..epsilon.R.alpha. protein by a linker). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding. . .

SUMM A preferred equine ***Fc*** .sub..epsilon.R.alpha. protein of the

present invention is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the following nucleic acid molecules: neqFc.sub..epsilon.R.alpha..sub.1015, neqFc.sub..epsilon.R.alpha..sub.765, neqFc.sub..epsilon.R.alpha..sub.708 and neqFc.sub..epsilon.R.alpha..sub.603. Preferably, the equine ***Fc*** .sub..epsilon.R.alpha. protein binds to IgE. A further preferred isolated protein is encoded by a nucleic acid molecule that hybridizes under stringent. . .

SUMM . . . acid sequences reported in GenBank.TM. indicates that SEQ ID NO:2 showed the most homology, i.e., about 61% identity, with a ***human*** high affinity IgE receptor .alpha.-subunit (SwissProt accession number P12319).

SUMM More preferred equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention include proteins comprising amino acid sequences that are at least about 65%, preferably at least. . .

SUMM More preferred equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention include proteins encoded by a nucleic acid molecule comprising at least a portion of neqFc.sub..epsilon.R.alpha..sub.1015,. . . allelic variants of such nucleic acid molecules, the portion being capable of binding to IgE. More preferred is an equine ***Fc*** .sub..epsilon.R.alpha. protein encoded by neqFc.sub..epsilon.R.alpha..sub.1015, neqFc.sub..epsilon.R.alpha..sub.765, neqFc.sub..epsilon.R.alpha..sub.708 and/or neqFc.sub..epsilon.R.alpha..sub.603, or by an allelic variant of such nucleic acid molecules. Particularly preferred equine ***Fc*** .sub..epsilon.R.alpha. proteins are PequFc.sub..epsilon.R.alpha..sub.255, PequFc.sub..epsilon.R.alpha..sub.236 and PequFc.sub..epsilon.R.alpha..sub.201.

SUMM In one embodiment, a preferred equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention is encoded by at least a portion of SEQ ID NO:1, SEQ ID NO:4, SEQ. . .

SUMM Also preferred is an equine ***Fc*** .sub..epsilon.R.alpha. protein encoded by an allelic variant of a nucleic acid molecule comprising at least a portion of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:6 and/or SEQ ID NO:11. Particularly preferred equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention include SEQ ID NO:2, SEQ ID NO:7 and SEQ ID NO:12 (including, but not limited. . .

SUMM . . . embodiment of the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with an equine ***Fc*** .sub..epsilon.R.alpha. gene. The identifying characteristics of such a gene are heretofore described. A nucleic acid molecule of the present invention can include an isolated natural equine ***Fc*** .sub..epsilon.R.alpha. gene or a homolog thereof, the latter of which is described in more detail below. A nucleic acid molecule of. . . nucleic acid molecule of the present invention is the minimal size that can form a stable hybrid with an equine ***Fc*** .sub..epsilon.R.alpha. gene under stringent hybridization conditions.

SUMM . . . molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to ***human*** manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecule of the present invention can be isolated from its natural source or can be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules can include, for example, natural allelic variants and nucleic acid molecules modified by nucleotide insertions, deletions, substitutions,. . . a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention or to form stable hybrids under stringent conditions with natural gene isolates.

SUMM An equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecule homolog can be produced using a number of methods known to those skilled in the art (see,. . . mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with an equine ***Fc*** .sub..epsilon.R.alpha. gene or by screening for function of a protein encoded by the nucleic acid molecule (e.g., ability of an equine ***Fc*** .sub..epsilon.R.alpha. protein to bind equine IgE).

SUMM . . . isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers. . . interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding an equine ***Fc*** .sub..epsilon.R.alpha. protein.

SUMM One embodiment of the present invention is an equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecule that hybridizes under stringent hybridization conditions with nucleic acid molecule neqFc.sub..epsilon.R.alpha..sub.1015 and preferably with a nucleic acid. . .

SUMM . . . acid sequences reported in GenBank indicates that SEQ ID NO:1 showed the most homology, i.e., about 75% identity to a ***human*** mRNA for immunoglobulin E receptor alpha chain gene (Accession number X06948).

SUMM Preferred equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules include nucleic acid molecules having a nucleic acid sequence that is at least about 80%, preferably at. . .

SUMM . . . ID NO:5, SEQ ID NO:6, SEQ ID NO:8 and/or SEQ ID NO:11, that is capable of hybridizing to an equine ***Fc*** .sub..epsilon.R.alpha. gene of the present invention, as well as allelic variants thereof. A more preferred nucleic acid molecule includes the nucleic. . .

SUMM Preferred equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules also include nucleic acid molecules having a nucleic acid sequence that is at least about 80%, preferably. . .

SUMM Knowing the nucleic acid sequences of certain equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of. . . (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules from other horses. Such nucleic acid molecules can be obtained in a variety of ways including screening. . .

SUMM . . . conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising equine ***Fc*** .sub..epsilon.R.alpha. genes or other equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of. . . for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules or therapeutic reagents to inhibit equine ***Fc*** .sub..epsilon.R.alpha. protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also includes the. . .

SUMM . . . is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulation of equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules of the present invention.

SUMM . . . the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences. . .

SUMM . . . that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecules disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include neqFc.sub..epsilon.R.alpha..sub.1015, neqFc.sub..epsilon.R.alpha..sub.765, neqFc.sub..epsilon.R.alpha..sub.708 . . .

SUMM . . . production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention or can be capable of producing such proteins after being transformed with at least one. . . cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., ***human*** , murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK.sup.31 cells and/or. . .

SUMM Isolated equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins,. . . permit protein production. An effective medium refers to any medium in which a cell is cultured to produce an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention. Such a medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources,. . .

SUMM The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention or a mimetope thereof (i.e., anti-equine ***Fc*** .sub..epsilon.R.alpha. antibodies). As used herein, the term "selectively binds to" an equine ***Fc*** .sub..epsilon.R.alpha. protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof. . . in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., ibid. An anti-equine ***Fc*** .sub..epsilon.R.alpha. antibody preferably selectively binds to an equine ***Fc*** .sub..epsilon.R.alpha. protein in such a way as to reduce the activity of that protein.

SUMM . . . antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not. . .

SUMM . . . are within the scope of the present invention. For example, such antibodies can be used (a) as tools to detect ***Fc*** ***epsilon*** ***receptor*** in the presence or absence of IgE and/or (b) as tools to screen expression libraries and/or to recover desired proteins. . . proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target cytotoxic agents to cells having ***Fc*** ***epsilon*** ***receptors*** such as those disclosed herein in order to directly kill such cells. Targeting can be accomplished by conjugating (i.e., stably. . . in the art. Suitable cytotoxic agents are known to those skilled in the art. Antibodies of the present invention, including ***Fc*** .sub..epsilon.R.alpha.-binding portions thereof, can also be used, for example, to inhibit binding of IgE to ***Fc*** ***epsilon*** ***receptors*** , to produce anti-equine ***Fc*** .sub..epsilon.R.alpha. idiotypic antibodies, to purify cells having equine ***Fc*** .sub..epsilon.R.alpha. proteins, to stimulate intracellular signal transduction through an equine ***Fc*** .sub..epsilon.R.alpha. and to identify cells having equine ***Fc*** .sub..epsilon.R.alpha. proteins.

SUMM An equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention can include chimeric molecules comprising a portion of an equine ***Fc*** .sub..epsilon.R.alpha. molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the ***Fc*** .sub..epsilon.R.alpha. molecule portion binds to IgE in essentially the same manner as a ***Fc*** .sub..epsilon.R.alpha. molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of an. . .

SUMM An equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention can include chimeric molecules comprising a portion of an equine ***Fc*** .sub..epsilon.R.alpha. molecule that binds to an IgE and a second molecule, such as an enzyme, that enables the chimeric molecule to bind to IgE in essentially the same manner as a ***Fc*** .sub..epsilon.R.alpha. molecule which does not include such a second molecule, and to hydrolyze a substrate in such a manner so as. . . An example of a suitable second molecule includes alkaline phosphatase, horse radish peroxidase or urease. In one embodiment an equine ***Fc*** .sub..epsilon.R.alpha. chimeric molecule of the present invention comprises a protein encoded by a recombinant molecule comprising a nucleic acid molecule that encodes at least a portion of an equine ***Fc*** .sub..epsilon.R.alpha. molecule that binds to an IgE, operatively linked to a nucleic acid molecule that encodes an enzyme, preferably alkaline phosphatase.

SUMM An equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention can be contained in a formulation, herein referred to as a ***Fc*** .sub..epsilon.R.alpha. molecule formulation. For example, an

equine ***Fc*** .sub..epsilon.R.alpha. molecule can be combined with a buffer in which the equine ***Fc*** .sub..epsilon.R.alpha. molecule is solubilized, and/or with a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which an equine ***Fc*** .sub..epsilon.R.alpha. molecule can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate. . . not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be mixed with equine ***Fc*** .sub..epsilon.R.alpha. molecules or conjugated (i.e., attached) to equine ***Fc*** .sub..epsilon.R.alpha. molecules in such a manner as to not substantially interfere with the ability of the equine ***Fc*** .sub..epsilon.R.alpha. molecules to selectively bind to IgE.

SUMM An equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention can be bound to the surface of a cell comprising the equine ***Fc*** .sub..epsilon.R.alpha. protein. A preferred equine ***Fc*** .sub..epsilon.R.alpha. protein-bearing cell includes a recombinant cell comprising a nucleic acid molecule encoding an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention. A more preferred recombinant cell of the present invention comprises a nucleic acid molecule that. . .

SUMM In addition, an equine ***Fc*** .sub..epsilon.R.alpha. molecule formulation of the present invention can include not only an equine ***Fc*** .sub..epsilon.R.alpha. molecule but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers. . .

SUMM . . . of the present invention is a method to detect IgE which includes the steps of: (a) contacting an isolated equine ***Fc*** .sub..epsilon.R.alpha. molecule with a putative IgE-containing composition under conditions suitable for formation of an equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex; and (b) detecting the presence of IgE by detecting the equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex. Presence of such an equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex indicates that the animal is producing IgE. Preferred IgE to detect using an equine ***Fc*** .sub..epsilon.R.alpha. molecule include equine IgE, canine IgE, feline IgE and ***human*** IgE, with equine IgE being particularly preferred. The present method can further include the step of determining whether an IgE complexed with an equine ***Fc*** .sub..epsilon.R.alpha. protein is heat labile. Preferably, a heat labile IgE is determined by incubating an IgE at about 56.degree. C. for. . .

SUMM . . . used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with an equine ***Fc*** .sub..epsilon.R.alpha. molecule. Formation of a complex between an equine ***Fc*** .sub..epsilon.R.alpha. molecule and an IgE refers to the ability of the equine ***Fc*** .sub..epsilon.R.alpha. molecule to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between an equine ***Fc*** .sub..epsilon.R.alpha. molecule and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures,. . .

SUMM . . . are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between equine ***Fc*** .sub..epsilon.R.alpha. molecule and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in. . .

SUMM . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the equine ***Fc*** .sub..epsilon.R.alpha. molecule or to a reagent that selectively binds to the equine ***Fc*** .sub..epsilon.R.alpha. molecule or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable. . . compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin available from Pierce, Rockford, Ill.). According to the present invention, a ***detectable***

marker can be connected to an equine ***Fc*** .sub..epsilon.R.alpha. molecule using, for example, chemical conjugation or recombinant DNA technology (e.g., connection of a fusion segment such as that described. . . binding domain; an immunoglobulin binding; a sugar binding domain; and a "tag" domain). Preferably a carbohydrate group of the equine ***Fc*** .sub..epsilon.R.alpha. molecule is chemically conjugated to biotin.

SUMM In one embodiment, a complex is detected by contacting a putative IgE-containing composition with an equine ***Fc*** .sub..epsilon.R.alpha. molecule that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to an equine ***Fc*** .sub..epsilon.R.alpha. molecule includes, but is not limited to, a radioactive label, a fluorescent label, an enzyme label, a chemiluminescent label, a chromophoric label or a ligand. A ***detectable*** ***marker*** is conjugated to an equine ***Fc*** .sub..epsilon.R.alpha. molecule in such a manner as not to block the ability of the equine ***Fc*** .sub..epsilon.R.alpha. molecule to bind to the IgE being detected. Preferably, a carbohydrate group of an equine ***Fc*** .sub..epsilon.R.alpha. molecule is conjugated to biotin.

SUMM In another embodiment, an equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex is detected by contacting a putative IgE-containing composition with an equine ***Fc*** .sub..epsilon.R.alpha. molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the equine ***Fc*** .sub..epsilon.R.alpha. molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, an antigen, an antibody and a lectin, depending upon which portion of the equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex is being detected. Preferred indicator molecules that are antibodies include, for example, anti-IgE antibodies and anti-equine ***Fc*** .sub..epsilon.R.alpha. antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention produced in insect cells. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

SUMM In one preferred embodiment, an equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex is detected by contacting the complex with an indicator molecule that selectively binds to an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention. Examples of such indicator molecule includes, but are not limited to, an antibody that selectively binds to an equine ***Fc*** .sub..epsilon.R.alpha. molecule (referred to herein as an anti-equine ***Fc*** .sub..epsilon.R.alpha. antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to an equine ***Fc*** .sub..epsilon.R.alpha. molecule. An equine ***Fc*** .sub..epsilon.R.alpha. molecule conjugated to biotin is preferably detected using streptavidin.

SUMM In another preferred embodiment, an equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex is detected by contacting the complex with indicator molecule that selectively binds to an IgE antibody (referred to. . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., a ***Fc*** receptor), and an antibody-binding complement protein. A preferred indicator molecule includes an anti-equine IgE antibody. As used herein, an anti-IgE. . .

SUMM . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

SUMM A preferred immunoabsorbent assay method includes a step of either: (a) immobilizing an equine ***Fc*** .sub..epsilon.R.alpha. molecule on a substrate prior to contacting an equine ***Fc*** .sub..epsilon.R.alpha. molecule with a putative IgE-containing composition to form an equine ***Fc*** .sub..epsilon.R.alpha. molecule-immobilized substrate; and (b) binding a putative IgE-containing composition on a substrate prior to contacting an equine ***Fc*** .sub..epsilon.R.alpha. molecule with a putative IgE-containing

composition to form a putative IgE-containing composition-bound substrate. Preferably, the substrate includes a non-coated substrate, an equine ***Fc*** .sub..epsilon.R.alpha. molecule-immobilized substrate, an antigen-immobilized substrate or an anti-IgE antibody-immobilized substrate.

SUMM . . . whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention is used as a capture molecule when the equine ***Fc*** .sub..epsilon.R.alpha. molecule is bound on a substrate. Alternatively, an equine ***Fc*** .sub..epsilon.R.alpha. molecule is used as an indicator molecule when the equine ***Fc*** .sub..epsilon.R.alpha. molecule is not bound on a substrate. Suitable molecules for use as capture molecules or indicator molecules include, but are not limited to, an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

SUMM . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

SUMM . . . the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is an equine ***Fc*** .sub..epsilon.R.alpha. molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

SUMM In one embodiment, an equine ***Fc*** .sub..epsilon.R.alpha. molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or. . . biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the equine ***Fc*** .sub..epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the equine ***Fc*** .sub..epsilon.R.alpha. molecule:IgE complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . .

SUMM . . . if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. An equine ***Fc*** .sub..epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the equine ***Fc*** .sub..epsilon.R.alpha. molecule and the anti-IgE antibody:IgE complex. Preferably, the equine ***Fc*** .sub..epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess equine ***Fc*** .sub..epsilon.R.alpha. molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

SUMM . . . non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. An equine ***Fc*** .sub..epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the equine ***Fc*** .sub..epsilon.R.alpha. molecule and the IgE. Preferably, the equine ***Fc*** .sub..epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess equine ***Fc*** .sub..epsilon.R.alpha. molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

SUMM . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . . which is directed downstream by the

flow path. The capture zone contains the capture reagent, in this case an equine ***Fc*** .sub..epsilon.R.alpha. molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture reagent is. . .

SUMM . . . used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising an equine ***Fc*** .sub..epsilon.R.alpha. molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a capture. . .

SUMM . . . in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention and an isolated IgE known to bind to the equine ***Fc*** .sub..epsilon.R.alpha. molecule. The absence of binding of the equine ***Fc*** .sub..epsilon.R.alpha. molecule to the known IgE indicates the presence of IgE in the putative IgE-containing composition. The known IgE is preferably conjugated to a ***detectable*** ***marker*** .

SUMM . . . IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising an equine ***Fc*** .sub..epsilon.R.alpha. protein and a means for detecting an IgE. Suitable and preferred equine ***Fc*** .sub..epsilon.R.alpha. protein are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the equine ***Fc*** .sub..epsilon.R.alpha. protein or to an IgE. A preferred kit of the present invention further comprises a detection means including one or. . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to an equine ***Fc*** .sub..epsilon.R.alpha. protein (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including equines, canines and/or felines.

SUMM . . . invention is a general allergen kit comprising an allergen common to all regions of the United States and an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that are. . .

SUMM . . . allergen including wheat, corn, alfalfa, hay, straw, oats, grains, processed grain by-products and grasses and/or dusts thereof, and an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention. Kits for detecting hypersensitivity to feeds and/or feed dust allergens can be used in combination. . . .

SUMM . . . present invention is a therapeutic composition that, when administered to an animal in an effective manner, is capable of reducing ***Fc*** receptor mediated reactions associated with diseases related to biological responses involving ***Fc*** receptor function. A therapeutic composition of the present invention can include: an isolated equine ***Fc*** .sub..epsilon.R.alpha. protein, or homolog thereof; a mimetope of an equine ***Fc*** .sub..epsilon.R.alpha. protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with an equine ***Fc*** .sub..epsilon.R.alpha. gene; an isolated antibody that selectively binds to an equine ***Fc*** .sub..epsilon.R.alpha. protein; and/or an inhibitor that interferes with formation of a complex between an equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE.

SUMM One embodiment of a therapeutic composition of the present invention is a therapeutic compound comprising an equine ***Fc*** .sub..epsilon.R.alpha. molecule of the present invention, that binds to an IgE. According to the present invention, an equine ***Fc*** .sub..epsilon.R.alpha. molecule competes for IgE with naturally-occurring ***Fc*** ***epsilon*** ***receptors*** , particularly those on mastocytoma cells, mast cells or basophils, so that IgE is bound to the administered equine ***Fc*** .sub..epsilon.R.alpha. molecule and thus is unable to bind to ***Fc*** ***epsilon*** ***receptor*** on a cell, thereby inhibiting mediation of a biological response. Preferred equine ***Fc*** .sub..epsilon.R.alpha. molecule for use in a therapeutic composition comprises an equine ***Fc*** .sub..epsilon.R.alpha. protein, or homolog thereof, as described herein, particularly a fragment thereof, which binds to IgE. Equine ***Fc*** .sub..epsilon.R.alpha. molecules for use in a therapeutic composition can be in a monovalent and/or

multivalent form, so long as the equine ***Fc*** .sub..epsilon.R.alpha. molecule is capable of binding to IgE. A more preferred equine ***Fc*** .sub..epsilon.R.alpha. molecule for use in a therapeutic composition includes a soluble fragment of an equine ***Fc*** .sub..epsilon.R.alpha. protein. A preferred equine ***Fc*** .sub..epsilon.R.alpha. protein is encoded by neqFc.sub..epsilon.R.alpha..sub.603 and an even more preferred equine ***Fc*** .sub..epsilon.R.alpha. protein is PequFc.sub..epsilon.R.alpha..sub.201.

SUMM . . . therapeutic composition of the present invention comprises a therapeutic compound that interferes with the formation of a complex between equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE, usually by binding to or otherwise interacting with or otherwise modifying the equine ***Fc*** .sub..epsilon.R.alpha. protein's IgE binding site. Equine ***Fc*** .sub..epsilon.R.alpha. protein inhibitors can also interact with other regions of the equine ***Fc*** .sub..epsilon.R.alpha. protein to inhibit equine ***Fc*** .sub..epsilon.R.alpha. protein activity, for example, by allosteric interaction. An inhibitor of an equine ***Fc*** .sub..epsilon.R.alpha. protein can interfere with ***Fc*** .sub..epsilon.R.alpha. protein and IgE complex formation by, for example, preventing formation of a ***Fc*** .sub..epsilon.R.alpha. protein and IgE complex or disrupting an existing ***Fc*** .sub..epsilon.R.alpha. protein and IgE complex causing the ***Fc*** .sub..epsilon.R.alpha. protein and IgE to dissociate. An inhibitor of an equine ***Fc*** .sub..epsilon.R.alpha. protein is usually a relatively small molecule. Preferably, an equine ***Fc*** .sub..epsilon.R.alpha. protein inhibitor of the present invention is identified by its ability to bind to, or otherwise interact with, an equine ***Fc*** .sub..epsilon.R.alpha. protein, thereby interfering with the formation of a complex between an equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE.

SUMM Preferred inhibitors of an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention include, but are not limited to, a substrate analog of an equine ***Fc*** .sub..epsilon.R.alpha. protein, a mimetope of an equine ***Fc*** .sub..epsilon.R.alpha. protein, a soluble (i.e., secreted form of an equine ***Fc*** .sub..epsilon.R.alpha. protein) portion of an equine ***Fc*** .sub..epsilon.R.alpha. protein that binds to IgE, and other molecules that bind to an equine ***Fc*** .sub..epsilon.R.alpha. protein (e.g., to an allosteric site) in such a manner that IgE-binding activity of the equine ***Fc*** .sub..epsilon.R.alpha. protein is inhibited. An equine ***Fc*** .sub..epsilon.R.alpha. protein substrate analog refers to a compound that interacts with (e.g., binds to, associates with, modifies) the IgE-binding site of an equine ***Fc*** .sub..epsilon.R.alpha. protein. A preferred equine ***Fc*** .sub..epsilon.R.alpha. protein substrate analog inhibits IgE-binding activity of an equine ***Fc*** .sub..epsilon.R.alpha. protein. Equine ***Fc*** .sub..epsilon.R.alpha. protein substrate analogs can be of any inorganic or organic composition, and, as such, can be, but are not limited to, peptides, nucleic acids, and peptidomimetic compounds. Equine ***Fc*** .sub..epsilon.R.alpha. protein substrate analogs can be, but need not be, structurally similar to an equine ***Fc*** .sub..epsilon.R.alpha. protein's natural substrate (e.g., IgE) as long as they can interact with the active site (e.g., IgE-binding site of that equine ***Fc*** .sub..epsilon.R.alpha.). Equine ***Fc*** .sub..epsilon.R.alpha. protein substrate analogs can be designed using computer-generated structures of equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention or computer structures of, for example, the ***Fc*** domain of IgE. Substrate analogs can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides, peptidomimetic. . . . inorganic or organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., an equine ***Fc*** .sub..epsilon.R.alpha. protein or anti-equine ***Fc*** .sub..epsilon.R.alpha. idiotypic antibody). A preferred equine ***Fc*** .sub..epsilon.R.alpha. protein substrate analog is a peptidomimetic compound (i.e., a compound that is structurally and/or functionally similar to a natural substrate of an equine ***Fc*** .sub..epsilon.R.alpha. protein of the present invention, particularly to the region of the substrate that binds to an equine ***Fc***

.sub..epsilon.R.alpha. protein, but that inhibits IgE binding upon interacting with the IgE binding site).

SUMM Equine ***Fc*** .sub..epsilon.R.alpha. molecules, as well as other inhibitors and therapeutic compounds, can be used directly as compounds in compositions of the present. . .

SUMM In one embodiment, a therapeutic composition of the present invention can be used to reduce a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response in an animal by administering such a composition to an animal. Preferably, an animal is treated by administering. . . therapeutic composition of the present invention in such a manner that a therapeutic compound (e.g., an inhibitor of an equine ***Fc*** .sub..epsilon.R.alpha. protein, an anti-equine ***Fc*** .sub..epsilon.R.alpha. antibody, an inhibitor of IgE, or nucleic acid molecules encoding equine ***Fc*** .sub..epsilon.R.alpha. proteins) binds to an IgE or a ***Fc*** ***epsilon*** ***receptor*** in the animal. Such administration could be by a variety of routes known to those skilled in the art including,. . .

SUMM Compositions of the present invention can be administered to any animal having a ***Fc*** ***epsilon*** ***receptor*** or an IgE that binds to a therapeutic compound of the present invention or to a protein expressed by a. '. .

SUMM . . . a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, ***human*** serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

SUMM . . . the blood of an animal at a constant rate sufficient to attain therapeutic dose levels of the composition to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in the animal. As used herein, a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response refers to cellular responses that occur when ***Fc*** ***epsilon*** ***receptor*** is complexed with IgE. For example, a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response includes release of biological mediators, such as histamine, prostaglándins and/or proteases, that can trigger clinical symptoms of allergy.. . .

SUMM . . . of skill in the art in accordance with the given condition of a patient. For example, to regulate an antigen-specific ***Fc*** ***epsilon*** ***receptor*** -mediated response, a therapeutic composition may be administered more frequently when an antigen is present in a patient's environment in high. . .

SUMM . . . can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into an equine ***Fc*** .sub..epsilon.R.alpha. protein or an equine ***Fc*** .sub..epsilon.R.alpha. RNA (e.g., antisense RNA, ribozyme, triple helix forms or RNA drug) in the animal. Nucleic acid molecules can be delivered. . .

SUMM : . . the recipient animal and directs the production of a protein or RNA nucleic acid molecule that is capable of reducing ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in the animal. For example, a recombinant virus comprising an equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecule of the present invention is administered according to a protocol that results in the animal producing an amount of protein or RNA sufficient to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. A preferred single dose of a recombinant virus of the present invention is from about 1.times.10.sup.4 to about. . .

SUMM . . . a therapeutic composition of the present invention includes recombinant cells of the present invention that comprises at least one equine ***Fc*** .sub..epsilon.R.alpha. of the present invention. Preferred recombinant cells for this embodiment include Salmonella, E. coli, Listeria, Mycobacterium, S. frugiperda, yeast, (including. . .

SUMM . . . an animal an effective amount of a therapeutic composition selected from the group consisting of an inhibitor of an equine ***Fc*** .sub..epsilon.R.alpha. and an equine ***Fc*** .sub..epsilon.R.alpha. protein (including homologs), wherein said equine ***Fc*** .sub..epsilon.R.alpha. is capable of binding to IgE. Suitable therapeutic compositions and methods of administration methods are disclosed herein. According to the. . . invention, a therapeutic composition and method of the present invention can be used to prevent or alleviate symptoms associated with ***Fc*** ***epsilon***

receptor -mediated biological responses.

SUMM The efficacy of a therapeutic composition of the present invention to effect ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses can be tested using standard methods for detecting ***Fc*** receptor-mediated immunity including, but not limited to, immediate hypersensitivity, delayed hypersensitivity, antibody-dependent cellular cytotoxicity (ADCC), immune complex activity, mitogenic activity,. . .

SUMM An inhibitor of equine ***Fc*** .sub..epsilon.R.alpha. activity can be identified using equine ***Fc*** .sub..epsilon.R.alpha. proteins of the present invention by determining the ability of an inhibitor to prevent or disrupt complex formation between an equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE. One embodiment of the present invention is a method to identify a compound capable of inhibiting equine ***Fc*** .sub..epsilon.R.alpha. activity. Such a method includes the steps of (a) contacting (e.g., combining, mixing) an isolated equine ***Fc*** .sub..epsilon.R.alpha. protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the equine ***Fc*** .sub..epsilon.R.alpha. protein has IgE binding activity, and (b) determining if the putative inhibitory compound inhibits the IgE binding activity. Putative inhibitory. . .

SUMM The present invention also includes a test kit to identify a compound capable of inhibiting equine ***Fc*** .sub..epsilon.R.alpha. activity. Such a test kit includes: an isolated equine ***Fc*** .sub..epsilon.R.alpha. protein having IgE binding activity or a complex of equine ***Fc*** .sub..epsilon.R.alpha. protein and IgE; and a means for determining the extent of inhibition of IgE binding activity in the presence of. . .

DETD This example describes the isolation, by DNA hybridization, of a nucleic acid molecule encoding a ***Fc*** .sub..epsilon.R.alpha. chain from Equus caballus.

DETD . . . was isolated from a horse buffy coat cDNA library by its ability to hybridize with a .sup.32P-labeled cDNA encoding the ***human*** ***Fc*** .sub..epsilon.R.alpha. chain (Kochan et al., Nucleic Acids Res. 16:3584, 1988). The horse buffy coat cDNA library was prepared as follows. Total. . .

DETD The horse buffy coat cDNA library was screened, using duplicate plaque filter lifts, with a .sup.32P-labeled cDNA encoding the ***human*** ***Fc*** .sub..epsilon.R.alpha. chain under the following conditions. The filters were pre-hybridized and hybridized in a hybridization solution including 5.times.SSC, 5.times.Denhardts, 0.5% SDS. . .

DETD This example describes the sequencing of an equine ***Fc*** .sub..epsilon.R.alpha. chain nucleic acid molecule of the present invention.

DETD . . . includes GenBank+EMBL+DDBJ+PDB. The highest scoring match of the homology search at the amino acid level was SwissProt accession number P12319: ***human*** high affinity IgE receptor .alpha.-chain, which was about 61% identical with SEQ ID NO:2. At the nucleotide level, the search was performed using SEQ ID NO:1, which was most similar to GenBank accession number X06948, ***human*** mRNA for immunoglobulin E receptor alpha chain, which was about 75% identical to SEQ ID NO:1.

DETD This Example demonstrates the production of an equine ***Fc*** .sub..epsilon.R.alpha. chain protein in eukaryotic cells.

DETD . . . of SEQ ID NO:1, operatively linked to baculovirus polyhedron transcription control sequences, was produced in the following manner. An equine ***Fc*** .sub..epsilon.R.alpha. nucleic acid molecule-containing fragment of about 603 nucleotides was PCR amplified from neqFc.sub..epsilon.R.alpha..sub.1015 using sense primer EqIgErFor having the nucleic. . .

DETD . . . molecule referred to herein as pFB-neqFc.sub..epsilon.R.alpha..sub.603. Translation of SEQ ID NO:11 indicates that the nucleic acid molecule neqFc.sub..epsilon.R.alpha..sub.603 encodes a ***Fc*** .sub..epsilon.R.alpha. protein of about 201 amino acids, referred to herein as PequFc.sub..epsilon.R.alpha..sub.201, having amino acid sequence SEQ ID NO:12.

DETD . . . frugiperda: pFB-neqFc.sub..epsilon.R.alpha..sub.603 can be cultured using conditions known to those skilled in the art in order to produce the equine ***Fc*** .sub..epsilon.R.alpha. protein, PequFc.sub..epsilon.R.alpha..sub.201 or a secreted form thereof.

CLM What is claimed is:

1. A composition comprising an excipient and an isolated equine

Fc .sub..epsilon.R.alpha. protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:7, SEQ ID. . .
2. The composition of claim 1, wherein said equine ***Fc*** .sub..epsilon.R.alpha. protein is a soluble equine ***Fc*** .sub..epsilon.R.alpha. protein that binds to IgE.

4. The composition of claim 1, wherein said isolated equine ***Fc*** .sub..epsilon.R.alpha. protein is encoded by a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of: SEQ. . .
5. The composition of claim 1, wherein said isolated equine ***Fc*** .sub..epsilon.R.alpha. protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:7, and SEQ. . .
6. An isolated equine ***Fc*** .sub..epsilon.R.alpha. protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:7, and SEQ. . .
7. An isolated equine ***Fc*** .sub..epsilon.R.alpha. protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:7, SEQ ID. . .

L13 ANSWER 11 OF 24 USPATFULL on STN
AN 2002:301238 USPATFULL
TI Detection of allergen-specific IgE
IN McCall, Catherine A., Boulder, CO, UNITED STATES
Babu, Uma Mahesh, Fort Collins, CO, UNITED STATES
Radecki, Steven V., Fort Collins, CO, UNITED STATES
PI US 2002168782 A1 20021114
AI US 2002-38519 A1 20020103 (10)
PRAI US 2001-325812P 20010928 (60)
US 2001-259450P 20010103 (60)
DT Utility
FS APPLICATION
LREP Heska Corporation, 1613 Prospect Parkway, Fort Collins, CO, 80525
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1208
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to point-of-care devices and methods to screen animals with suspected IgE-mediated allergic disease. The present invention provides a simple and rapid preliminary immunoassay screen, in which a defined mixture of clinically relevant allergens is used to capture allergen-specific IgE present in a sample, followed by a second binding reagent that selectively binds IgE. The present invention further provides methods for prescribing immunotherapy treatment in animals having IgE-mediated diseases. Devices and kits useful for carrying out the methods of the invention are also provided.
SUMM . . . include, for example, anti-IgE antibodies or IgE receptors or mimetopes of such molecules. Such reagents can be labeled with a ***detectable*** ***marker*** or a ***detectable*** ***marker*** can be separately added that binds or otherwise associates with the reagent such that detection is possible.
SUMM . . . capable of identifying a desired percent of the relevant population. For example, if the desired target is 90% of all ***human*** patients with IgE-mediated allergic disease, those skilled in the art would select a mixture of allergens that are collectively known. . .
SUMM . . . IgG. In a preferred embodiment, the second binding agent binds IgE with a specificity similar to that exhibited by the ***human*** IgE receptor alpha chain protein. In another embodiment, the second binding agent fails to bind IgG under conditions in which. . .
SUMM [0037] The second binding reagent may be a polyclonal or monoclonal antibody that selectively binds IgE, the ***Fc*** . ***epsilon*** . ***receptor*** 1 (***Fc*** .epsilon.R1) (as described in U.S. Pat. No. 5,945,294, which is incorporated herein by reference), IgE-binding allergens or any molecule which selectively. . . bind to mast cells and basophils. Preferred antibodies of the present invention have the characteristics of a mAb produced using ***human*** ***Fc*** .epsilon.RI.alpha.-affinity purified IgE as described herein.
SUMM [0042] Any indicator or detection reagent such as enzymes, fluorescent

molecules, radiolabels, or other ***detectable*** ***marker*** known to those skilled in the art can be used. Enzymes are particularly useful as indicator agents. If the label. . .

SUMM . . . buffers and detection reagents. Preferred detection reagents of the invention include monoclonal antibodies that specifically bind canine, feline, equine, and ***human*** IgE.

DETD . . . present in complex samples such as dog sera, PBMCs or possibly even blood. They work well in combination with the ***human*** ***Fc*** .epsilon.RI.alpha. to calculate total serum IgE. With appropriate standards, dog IgE levels could easily be measured and compared to various stages. . . cytometry studies could be performed by using these antibodies to map cell surface IgE and to check the validity of ***Fc*** .epsilon.RI.alpha. based results. In addition, the anti-canine epsilon chain mAbs may be used for immunotherapy for allergic dogs.

DETD [0070] Mouse anti-feline IgE mAbs, mouse anti-equine IgE mAbs, and mouse anti- ***human*** IgE mAbs are made in the same manner as above. The mAbs may also be prepared in animals other than. . .

DETD . . . which had been heated at 56.degree. C. for one hour prior to use. (This procedure is known to denature the IgE- ***Fc*** receptor binding region which allows one to determine which antibodies bind IgE in or near this region) The plates were. . .

DETD [0073] The ability of the anti-canine IgE monoclonal antibodies to bind canine or feline IgE bound by immobilized ***human*** ***Fc*** - ***epsilon*** ***receptor*** I-alpha chain, was tested using the following capture-assay protocol.

DETD [0074] The wells of a microtiter plate were coated with the extracellular domain of ***human*** ***Fc*** - ***epsilon*** ***receptor*** I-alpha chain protein (huFc.epsilon.RI.alpha.) (prepared as described in U.S. Pat. No. 5,945,294) (42.5 ng/well) in CBC buffer, and the. . .

DETD [0079] This example discloses a method for the purification of recombinantly produced equine ***FcR*** .epsilon.RI.alpha. (EqFc.epsilon.I.alpha.). Cultures expressing recombinant EqFcR.epsilon.RI.alpha. were produced as described in U.S. Pat. No. 6,057,127, and the supernatants collected and adjusted. . .

DETD . . . the plate was washed four times using Wash buffer and either biotinylated anti-equine IgE mAb H357 (10 ng), biotinylated, recombinant ***human*** ***Fc*** ***epsilon*** ***receptor*** I alpha chain (huFc.epsilon.RI.alpha.) (13 ng) (prepared as described in U.S. Pat. No. 5,945,294) or biotinylated recombinant equine ***Fc*** ***epsilon*** ***receptor*** alpha (EqFc.epsilon.RI.alpha.) (13 ng) (prepared as described in Example 7) was added to the wells. (All molecules were biotinylated following. . .

DETD . . . The data demonstrates the absorbence obtained when H-357 is used to detect IgE is similar to that observed when either ***human*** or equine ***Fc*** .epsilon.RI.alpha. is used.

DETD . . . The wells of a microtiter plate were coated, at 50 ng/well, with either equine IgE, canine IgE, feline IgE or ***human*** IgE in CBC buffer and the plate incubated overnight at 4.degree. C. Excess fluid was removed, 200 .mu.l of Assay. . . values shown and all values over 100 are considered positive.

TABLE 6

mAb H-357 (ng/well)	Coating Immunoglobulin Equine IgE	Canine IgE	Feline IgE	***Human*** IgE
20	2639	208	3	4
10	2567	180	6	0
5	2496	161	0	0
2.5	2077	134	0	0
1.25. . .				

DETD [0084] This example demonstrates mAb H-357 has a much greater specificity for equine IgE than for canine, feline or ***human*** IgE.

DETD . . . shown in Table 7. Background values have been subtracted from those shown in the Table.

TABLE 7

Fc .epsilon.RI.alpha.	Coating Immunoglobulin			
	Human	Canine	Feline	Equine
(ng/ml)	IgE	IgE	IgE	IgE
1300	1482	2286	2973	2123
650	2109	2409	3038	2108
325	1933	2327	3007	2128
162.5	1896. . .			

DETD . . . in Table 8. Background values have been subtracted from those shown in the Table 9.

TABLE 9

Fc .epsilon.RI.alpha.	Coating Immunoglobulin			
	Human	Canine	Feline	Equine
(ng/ml)	IgE	IgE	IgE	IgE
1300	142	1805	2612	1838
650	173	1706	2663	1777
325	214	1733	2807	1834
162.5	112. . .			

DETD . . . The data demonstrates EqFc.epsilon.RI.alpha. binds canine, feline and equine IgE with high affinity but has a much lower affinity for ***human*** IgE.

CLM What is claimed is:
5. The device of claim 1, wherein the animal is a dog, cat, horse or ***human*** .

15. The method of claim 14, wherein the labeled reagent is an anti-canine IgE, anti-feline IgE, anti-equine IgE or anti- ***human*** IgE monoclonal antibody.

L13 ANSWER 12 OF 24 USPATFULL on STN
AN 2002:60937 USPATFULL
TI Method to detect IgE
IN Frank, Glenn R., Wellington, CO, UNITED STATES
Porter, James P., Fort Collins, CO, UNITED STATES
Rushlow, Keith E., Fort Collins, CO, UNITED STATES
Wassom, Donald L., Fort Collins, CO, UNITED STATES
PI US 2002034771 A1 20020321
US 6682894 B2 20040127
AI US 2001-944277 A1 20010830 (9)
RLI Division of Ser. No. US 1999-285873, filed on 31 Mar 1999, GRANTED, Pat. No. US 6309832 Division of Ser. No. US 1996-756387, filed on 26 Nov 1996, GRANTED, Pat. No. US 5945294
DT Utility
FS APPLICATION
LREP Heska Corporation, Intellectual Property Dept., 1613 Prospect Parkway, Fort Collins, CO, 80525
CLMN Number of Claims: 105
ECL Exemplary Claim: 1
DRWN 11 Drawing Page(s)
LN.CNT 2278
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention includes a method to detect IgE using a ***human*** ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon.R) to detect IgE antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

AB The present invention includes a method to detect IgE using a ***human*** ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon.R) to detect IgE antibodies in a biological sample from a cat, a dog, or a horse. The present invention also. . .

SUMM [0003] Until the discovery of the present invention, detection of IgE in samples obtained from non- ***human*** animals has been hindered by the absence of suitable reagents for detection of IgE. Various compounds have been used to. . . with other antibody idiotypes, such as gamma

isotype antibodies. The discovery of the present invention includes the use of a ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon.R) molecule to detect the presence of IgE in a putative IgE-containing composition. A ***Fc*** .sub..epsilon.R molecule provides an advantage over, for example anti-IgE antibodies, to detect IgE because a ***Fc*** .sub..epsilon.R molecule can bind to an IgE with more specificity (i.e., less idiotype cross-reactivity) and more sensitivity (i.e., affinity) than anti-IgE. . .

SUMM [0004] Lowenthal et al., 1993, Annals of Allergy 71:481-484, dog serum can transfer cutaneous reactivity to a ***human*** . While it is possible that Lowenthal et al. properly teach the binding of ***human*** ***Fc*** .sub..epsilon.R to canine IgE. Lowenthal et al., however, do not provide data defining the particular cellular proteins responsible for the transfer. . . by Lowenthal et al. is merely an interpretation. In addition, Lowenthal et al. do not teach the use of purified ***human*** ***Fc*** .sub..epsilon.R to detect canine IgE. The subunits of ***human*** ***Fc*** .sub..epsilon.R have been known as early as 1988 and have never been used to detect canine, feline or equine IgE. Indeed, U.S. Pat. No. 4,962,035, to Leder et al., issued Oct. 9, 1990, discloses ***human*** ***Fc*** .sub..epsilon.R but does not disclose the use of such a ***human*** ***Fc*** .sub..epsilon.R to detect ***human*** or non- ***human*** IgE. The use of purified ***human*** ***Fc*** .sub..epsilon.R avoids complications presented by use of ***Fc*** .sub..epsilon.R bound to a cell, such as non-specific binding of the ***Fc*** .sub..epsilon.R-bearing cell due to additional molecules present on the cell membrane. That purified ***human*** ***Fc*** .sub..epsilon.R detects non- ***human*** IgE is unexpected because inter-species binding between a ***Fc*** .sub..epsilon.R and an IgE is not predictable. For example, ***human*** ***Fc*** .sub..epsilon.R binds to rat IgE but rat ***Fc*** .sub..epsilon.R does not bind to ***human*** IgE.

SUMM [0005] The high affinity ***Fc*** .sub..epsilon.R consists of three protein chains, alpha, beta and gamma. Prior investigators have disclosed the nucleic acid sequence for: the alpha. . .

SUMM [0006] Thus, methods and kits are needed in the art that will provide specific detection of non- ***human*** IgE.

SUMM . . . that detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon.R molecule:IgE complex, wherein the IgE is selected from the group consisting of canine IgE, feline IgE and equine IgE; and (b) determining the presence of IgE by detecting the ***Fc*** .sub..epsilon.R molecule:IgE complex, the presence of the ***Fc*** .sub..epsilon.R molecule:IgE complex indicating the presence of IgE. A preferred ***Fc*** .sub..epsilon.R molecule in which a carbohydrate group of the ***Fc*** .sub..epsilon.R molecule is conjugated to biotin.

SUMM . . . conditions suitable for formation of a recombinant cell:IgE complex, in which the recombinant cell includes: a recombinant cell expressing a ***human*** ***Fc*** .sub..epsilon.R molecule; and a recombinant cell expressing an antibody that binds selectively to an IgE including canine IgE, feline IgE and. . .

SUMM . . . binding to the substrate; and (c) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with a ***Fc*** .sub..epsilon.R molecule. Preferably, the flea allergen is a flea saliva antigen and more preferably flea saliva products and/or flea saliva proteins.

SUMM . . . includes a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule and a means for detecting an IgE including canine IgE, feline IgE and equine IgE. Another embodiment is a general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule. Another embodiment is a kit for detecting flea allergy dermatitis comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule and a flea allergen.

SUMM [0011] Another embodiment of the present invention is an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc***

.sub..epsilon.R) alpha chain protein, in which a carbohydrate group of the ***Fc*** .sub..epsilon.R alpha chain protein is conjugated to biotin. A preferred ***Fc*** .sub..epsilon.R alpha chain protein comprises PhFc.sub..epsilon.R.alpha..sub.172-BIOT.

DRWD [0012] FIG. 1 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect canine IgE antibodies.

DRWD [0013] FIG. 2 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect plant allergen-specific canine IgE antibodies.

DRWD [0014] FIG. 3 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect ***human*** or canine IgE antibodies.

DRWD [0015] FIG. 4 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect flea allergen-specific canine IgE antibodies.

DRWD [0016] FIG. 5 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect flea allergen-specific and heartworm antigen-specific canine IgE antibodies.

DRWD [0017] FIG. 6 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect flea saliva-specific canine IgE antibodies.

DRWD [0018] FIG. 7 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect heartworm antigen-specific feline IgE antibodies.

DRWD [0019] FIG. 8 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect heartworm antigen-specific feline IgE antibodies.

DRWD [0020] FIG. 9 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect antigen-specific equine IgE antibodies.

DRWD [0021] FIG. 10 depicts ELISA results using basophilic leukemia cells expressing alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect canine IgE antibodies in sera from heartworm-infected dogs.

DRWD [0022] FIG. 11 depicts ELISA results using basophilic leukemia cells expressing alpha chain of ***human*** ***Fc*** .sub..epsilon.R to detect canine IgE antibodies in sera from flea saliva sensitized dogs.

DETD [0023] The present invention relates to the discovery that purified high affinity ***human*** ***Fc*** ***epsilon*** ***receptor*** (i.e., ***Fc*** .sub..epsilon.RI; referred to herein as ***Fc*** .sub..epsilon.R) can be used in certain non- ***human*** (i.e., canine, feline or equine) epsilon immunoglobulin (referred to herein as IgE or IgE antibody)-based detection (e.g., diagnostic, screening) methods and kits. The use of ***human*** ***Fc*** .sub..epsilon.R to detect non- ***human*** IgE is unexpected because canine, feline and equine immune systems are different from the ***human*** immune system, as well as from each other (i.e., molecules important to the immune system usually are species specific).

DETD [0024] One embodiment of the present invention is a method to detect a non- ***human*** IgE using an isolated ***human*** ***Fc*** .sub..epsilon.R molecule. It is to be noted that the term "a" entity or "an" entity refers to one or more of. . .

DETD [0025] According to the present invention, an isolated, or biologically pure, ***Fc*** .sub..epsilon.R molecule, is a molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the molecule has been purified. An isolated ***human*** ***Fc*** .sub..epsilon.R molecule of the present invention can be obtained from its natural source (e.g., from a ***human*** mast cell), can be produced using recombinant DNA technology or can be produced by chemical synthesis.

DETD [0026] A ***Fc*** .sub..epsilon.R molecule (also referred to herein as ***Fc*** .sub..epsilon.R or ***Fc*** .sub..epsilon.R protein) of the present invention can be a full-length protein, a portion of a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. A ***Fc*** .sub..epsilon.R molecule of the present invention can comprise a complete ***Fc*** .sub..epsilon.R (i.e., alpha, beta and gamma ***Fc*** .sub..epsilon.R chains), an alpha ***Fc*** .sub..epsilon.R chain (also referred to herein as ***Fc*** .sub..epsilon.R .alpha. chain) or portions thereof. Preferably, a ***Fc*** .sub..epsilon.R molecule comprises at least a portion of a ***Fc*** .sub..epsilon.R .alpha. chain that

binds to IgE, i.e., that is capable of forming an immunocomplex with an IgE constant region. Preferably, a ***Fc*** .sub..epsilon.R molecule of the present invention binds to IgE with an affinity of about K.sub.A.apprxeq.10.sup.8, more preferably with an affinity of. . .

DETD [0027] An isolated ***Fc*** .sub..epsilon.R molecule of the present invention, including a homolog, can be identified in a straight-forward manner by the ***Fc*** .sub..epsilon.R molecule's ability to form an immunocomplex with an IgE. Examples of ***Fc*** .sub..epsilon.R homologs include ***Fc*** .sub..epsilon.R proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted,. . .

DETD [0028] ***Fc*** .sub..epsilon.R homologs can be the result of natural allelic variation or natural mutation. ***Fc*** .sub..epsilon.R homologs of the present invention can also be produced using techniques known in the art including, but not limited to,. . .

DETD [0029] According to the present invention, a ***human*** ***Fc*** .sub..epsilon.R .alpha. chain of the present invention is encoded by at least a portion of the nucleic acid sequence of the coding strand of a cDNA encoding a full-length ***Fc*** .sub..epsilon.R .alpha. chain protein represented herein as SEQ ID NO:1, the portion at least encoding the IgE binding site of the ***Fc*** .sub..epsilon.R .alpha. chain protein. The double-stranded nucleic acid molecule including both the coding strand having SEQ ID NO:1 and the complementary. . . determined by one skilled in the art and is shown herein as SEQ ID NO:3) is referred to herein as ***Fc*** .sub..epsilon.R nucleic acid molecule nhFc.sub..epsilon.R.alpha..sub.1198. Translation of SEQ ID NO:1 suggests that nucleic acid molecule nhFc.sub..epsilon.R.alpha..sub.1198 encodes a full-length ***Fc*** .sub..epsilon.R .alpha. chain protein of about 257 amino acids, referred to herein as PhFc.sub..epsilon.R.alpha..sub.257, represented by SEQ ID NO:2, assuming an. . . skilled in the art to make modifications to the respective nucleic acid molecules and proteins to, for example, develop a ***Fc*** .sub..epsilon.R .alpha. chain protein with increased solubility and/or a truncated protein (e.g., a peptide) capable of detecting IgE, e.g., PhFc.sub..epsilon.R.alpha..sub.197 and PhFc.sub..epsilon.R.alpha..sub.172. Preferred ***Fc*** .sub..epsilon.R molecules include PhFc.sub..epsilon.R.alpha..sub.257, PhFc.sub..epsilon.R.alpha..sub.197, PhFc.sub..epsilon.R.alpha..sub.232 and PhFc.sub..epsilon.R.alpha..sub.172. Preferred nucleic acid molecules to encode a ***Fc*** .sub..epsilon.R molecules include nhFc.sub..epsilon.R.alpha..sub.774, nhFc.sub..epsilon.R.alpha..sub.1198, nhFc.sub..epsilon.R.alpha..sub.612, nhFc.sub..epsilon.R.alpha..sub.591, nhFc.sub..epsilon.R.alpha..sub.699 and/or nhFc.sub..epsilon.R.alpha..sub .516.

DETD [0030] Isolated ***Fc*** .sub..epsilon.R molecule protein of the present invention can be produced by culturing a cell capable of expressing the protein under conditions. . . Suitable and preferred nucleic acid molecules with which to transform a cell are as disclosed herein for suitable and preferred ***Fc*** .sub..epsilon.R nucleic acid molecules per se. Particularly preferred nucleic acid molecules to include in recombinant cells of the present invention include. . .

DETD . . . one nucleic acid molecule. Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing a ***Fc*** .sub..epsilon.R molecule protein of the present invention or can be capable of producing such proteins after being transformed with at least. . .

DETD . . . be transformed, examples of which are disclosed herein. A particularly preferred recombinant molecule includes pVL-nhFc.sub..epsilon.R.alpha..sub.612. Details regarding the production of ***Fc*** .sub..epsilon.R molecule nucleic acid molecule-containing recombinant molecules are disclosed herein. Particularly preferred recombinant cell of the present invention includes Trichoplusia ni-pVL-nhFc.sub..epsilon.R.alpha..sub.612.

DETD [0033] A ***Fc*** .sub..epsilon.R molecule of the present invention can include chimeric molecules comprising a portion of a ***Fc*** .sub..epsilon.R molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the ***Fc*** .sub..epsilon.R portion binds to IgE in essentially the same manner as a ***Fc*** .sub..epsilon.R molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of an. . .

DETD [0034] A ***Fc*** .sub..epsilon.R molecule of the present invention can be contained in a formulation, herein referred to as a ***Fc*** .sub..epsilon.R formulation. For example, a ***Fc*** .sub..epsilon.R can be combined with a buffer in which the ***Fc*** .sub..epsilon.R is solubilized, and/or a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a ***Fc*** .sub..epsilon.R can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate buffer,. . . not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be in mixed with ***Fc*** .sub..epsilon.R or conjugated (i.e., attached) to ***Fc*** .sub..epsilon.R in such a manner as to not substantially interfere with the ability of the ***Fc*** .sub..epsilon.R to selectively bind to IgE.

DETD [0035] A ***Fc*** .sub..epsilon.R of the present invention can be bound to the surface of a cell expressing the ***Fc*** .sub..epsilon.R. A preferred ***Fc*** .sub..epsilon.R-bearing cell includes a recombinant cell expressing a nucleic acid molecule encoding a ***human*** ***Fc*** .sub..epsilon.R alpha chain of the present invention. A more preferred recombinant cell of the present invention expresses a nucleic acid molecule. . .

DETD [0036] In addition, a ***Fc*** .sub..epsilon.R formulation of the present invention can include not only a ***Fc*** .sub..epsilon.R but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers to. . .

DETD [0038] The present invention also includes ***human*** ***Fc*** .sub..epsilon.R mimetopes and use thereof to detect IgE. In accordance with the present invention, a "mimetope" refers to any compound that is able to mimic the ability of a ***Fc*** .sub..epsilon.R molecule to bind to IgE. A mimetope can be a peptide that has been modified to decrease its susceptibility to. . . by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of ***Fc*** .sub..epsilon.R mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex technology, peptides identified by random screening of peptide libraries and proteins identified. . .

DETD [0039] One embodiment of the present invention is a method to detect non- ***human*** IgE which includes the steps of: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule with a putative IgE-containing composition under conditions suitable for formation of an ***Fc*** .sub..epsilon.R molecule:IgE complex; and (b) detecting levels of IgE by detecting said ***Fc*** .sub..epsilon.R molecule:IgE complex. Presence of such a ***Fc*** .sub..epsilon.R molecule:IgE complex indicates that the animal is producing IgE. Preferred non- ***human*** IgE to detect using a ***human*** ***Fc*** .sub..epsilon.R molecule include canine IgE, feline IgE and equine IgE. The present method can further include the step of determining whether an IgE complexed with a ***Fc*** .sub..epsilon.R molecule is heat labile. Methods to determine heat lability of IgE are disclosed in the Examples section. Preferably, an IgE. . . certain flea or heartworm allergens. Moreover, Applicants believe that identification of heat labile IgE and non-heat labile IgE using a ***human*** ***Fc*** .sub..epsilon.R suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE. As such, a ***Fc*** .sub..epsilon.R molecule of the present invention may be useful for detecting molecules bound by the ***Fc*** .sub..epsilon.R molecule but not identical to a known IgE.

DETD . . . As used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with a ***human*** ***Fc*** .sub..epsilon.R molecule. Formation of a complex between a ***Fc*** .sub..epsilon.R and an IgE refers to the ability of the ***Fc*** .sub..epsilon.R to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a ***Fc*** .sub..epsilon.R of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between a ***Fc*** .sub..epsilon.R and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures, reaction. . .

DETD . . . complexes are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between ***Fc*** .sub..epsilon.R and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in the. . .

DETD . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the ***Fc*** .sub..epsilon.R or to a reagent that selectively binds to the ***Fc*** .sub..epsilon.R or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable markers. . . biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin). Preferably, biotin is conjugated to an alpha chain of a ***Fc*** .sub..epsilon.R. Preferably a carbohydrate group of the ***Fc*** .sub..epsilon.R alpha chain is conjugated to biotin. A preferred ***Fc*** .sub..epsilon.R molecule conjugated to biotin comprises PhFc.sub..epsilon.R.alpha..sub.1 72-BIOT (the production of which is described in the Examples section).

DETD [0046] In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a ***Fc*** .sub..epsilon.R molecule that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a ***Fc*** .sub..epsilon.R molecule includes, but is not limited to, a radioactive label, a fluorescent label, a chemiluminescent label or a chromophoric label. A ***detectable*** ***marker*** is conjugated to a ***Fc*** .sub..epsilon.R molecule or a reagent in such a manner as not to block the ability of the ***Fc*** .sub..epsilon.R or reagent to bind to the IgE being detected. Preferably, a carbohydrate group of a ***Fc*** .sub..epsilon.R is conjugated to biotin.

DETD [0047] In another embodiment, a ***Fc*** .sub..epsilon.R molecule:IgE complex is detected by contacting a putative IgE-containing composition with a ***Fc*** .sub..epsilon.R molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the ***Fc*** .sub..epsilon.R molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, a ***Fc*** .sub..epsilon.R molecule, an antigen, an antibody and a lectin, depending upon which portion of the ***Fc*** .sub..epsilon.R molecule:IgE complex being detected. Preferred identifying labeled compounds that are antibodies include, for example, anti-IgE antibodies and anti- ***Fc*** .sub..epsilon.R antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a ***Fc*** .sub..epsilon.R molecule of the present invention produced in insect cells. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

DETD [0048] In one preferred embodiment, a ***Fc*** .sub..epsilon.R molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to a ***Fc*** .sub..epsilon.R molecule of the present invention. Examples of such a reagent includes, but are not limited to, an antibody that selectively binds to a ***Fc*** .sub..epsilon.R molecule (referred to herein as an anti- ***Fc*** .sub..epsilon.R antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to a ***Fc*** .sub..epsilon.R molecule. ***Fc*** .sub..epsilon.R molecules conjugated to biotin are preferably detected using streptavidin, more preferably using ImmunoPure.RTM. NeutrAvidin (available from Pierce, Rockford, Ill.).

DETD [0049] In another preferred embodiment, a ***Fc*** .sub..epsilon.R molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to an IgE antibody (referred to. . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., an ***Fc*** receptor), and an antibody-binding complement protein. Preferred anti-IgE reagents include, but are not limited to, D9, and CMI antibody #9,. . .

DETD . . . beads, latex beads, immunoblot membranes and immunoblot papers.

In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

DETD [0052] A preferred immunoabsorbent assay method includes a step of either: (a) binding an ***Fc*** .sub..epsilon.R molecule to a substrate prior to contacting a ***Fc*** .sub..epsilon.R molecule with a putative IgE-containing composition to form a ***Fc*** .sub..epsilon.R molecule-coated substrate; or (b) binding a putative IgE-containing composition to a substrate prior to contacting a ***Fc*** .sub..epsilon.R molecule with a putative IgE-containing composition to form a putative IgE-containing composition-coated substrate. Preferably, the substrate includes of a non-coated substrate, a ***Fc*** .sub..epsilon.R molecule-coated substrate, an antigen-coated substrate or an anti-IgE antibody-coated substrate.

DETD . . . upon whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, a ***Fc*** .sub..epsilon.R molecule of the present invention is used as a capture molecule when the ***Fc*** .sub..epsilon.R molecule is bound to a substrate. Alternatively, a ***Fc*** .sub..epsilon.R molecule is used as an indicator molecule when the ***Fc*** .sub..epsilon.R molecule is not bound to a substrate. Suitable molecule for use as capture molecules or indicator molecules include, but are not limited to, a ***Fc*** .sub..epsilon.R molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

DETD . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

DETD . . . molecule that can selectively bind to an IgE bound to the antigen, the indicator molecule can be conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label; to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . . and the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is a ***Fc*** .sub..epsilon.R molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

DETD [0056] In one embodiment, a ***Fc*** .sub..epsilon.R molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or. . . A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for ***Fc*** .sub..epsilon.R molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain ***Fc*** .sub..epsilon.R molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the ***Fc*** .sub..epsilon.R is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the ***Fc*** .sub..epsilon.R molecule:IgE complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . .

DETD . . . material, if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. A ***Fc*** .sub..epsilon.R molecule is added to the substrate and incubated to allow formation of a complex between the ***Fc*** .sub..epsilon.R molecule and the anti-IgE antibody:IgE complex. Preferably, the ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess ***Fc*** .sub..epsilon.R molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

DETD . . . Excess non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A ***Fc*** .sub..epsilon.R molecule is added to the substrate and incubated to allow formation of a complex between the ***Fc*** .sub..epsilon.R molecule and the IgE. Preferably, the ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable***

marker (preferably to biotin, an enzyme label or a fluorescent label). Excess ***Fc*** .sub..epsilon.R molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

DETD . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a calorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . . zone which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case a ***Fc*** .sub..epsilon.R molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture reagent is. . .

DETD . . . apparatus used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a ***Fc*** .sub..epsilon.R molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a capture. . .

DETD . . . assay in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to a ***Fc*** .sub..epsilon.R molecule of the present invention and an isolated IgE known to bind to the ***Fc*** .sub..epsilon.R molecule. The absence of binding of the ***Fc*** .sub..epsilon.R molecule to the known IgE indicating the presence of IgE in the putative IgE-containing composition.

DETD . . . detect IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule and a means for detecting an IgE including canine IgE, feline IgE and/or equine IgE. Suitable and preferred ***Fc*** .sub..epsilon.R molecules are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the ***Fc*** .sub..epsilon.R molecule or to an IgE. A preferred kit of the present invention further comprises a detection means including one or. . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a ***Fc*** .sub..epsilon.R molecule (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including canines, felines and/or equines.

DETD . . . present invention is a general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon.R molecule of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that are. . .

DETD . . . such as cod, halibut or and tuna, egg, milk, Brewer's yeast, whole wheat, corn, soybean, cheese and rice, and a ***human*** ***Fc*** .sub..epsilon.R molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

DETD [0070] This example describes the construction of a recombinant baculovirus expressing a truncated portion of the .alpha.-chain of the ***human*** ***Fc*** .sub..epsilon. receptor.

DETD [0071] Recombinant molecule pVL-nhFc.sub..epsilon.R.alpha..sub.612, containing a nucleic acid molecule encoding the extracellular domain of the ***Fc*** .sub..epsilon.R .alpha. chain, operatively linked to baculovirus polyhedron transcription control sequences was produced in the following manner. A cDNA clone encoding the full-length alpha chain (.alpha. chain) of the ***human*** ***Fc*** .sub..epsilon. receptor was obtained from Dr. Jean-Pierre Kinet (Harvard University, Cambridge, Mass.). The cDNA clone included an about 1198 nucleotide insert,. . . as SEQ ID NO: 1. Translation of SEQ ID NO: 1 indicates that nucleic acid molecule nhFc.sub..epsilon.R.alpha..sub.1198 encodes a full-length ***human*** ***Fc*** .sub..epsilon. receptor a chain protein of about 257 amino acids, referred to herein as PhFc.sub..epsilon.R.alpha..sub.257, having amino acid sequence SEQ ID. . . ID NO:1. The complement of SEQ ID NO:1 is represented herein by SEQ ID NO:3. The proposed mature protein (i.e., ***Fc*** .sub..epsilon.R.alpha. chain from which the signal sequence has been cleaved), denoted herein as PhFc.sub..epsilon.R.alpha..sub.232, contains about 232 amino acids which is. . .

DETD [0072] To produce a secreted form of the extracellular domain of the

Fc .sub..epsilon.R .alpha. chain, the hydrophobic transmembrane domain and the cytoplasmic tail of the ***Fc*** .sub..epsilon.R .alpha. chain encoded by nhFc.sub..epsilon.R.alpha..sub.1198 were removed as follows. A ***Fc*** .sub..epsilon.R .alpha. chain extracellular domain nucleic acid molecule-containing fragment of about 612 nucleotides was PCR amplified from nhFc.sub..epsilon.R.alpha..sub.11 98 using a forward. . . . EcoRI to produce nhFc.sub..epsilon.R.alpha..sub.612. Nucleic acid molecule nhFc.sub..epsilon.R.alpha..sub.612 contained an about 591 nucleotide fragment encoding the extracellular domain of the ***human*** ***Fc*** .sub..epsilon.R .alpha. chain, extending from about nucleotide 107 to about nucleotide 697 of SEQ ID NO 1, denoted herein as nucleic. . . . nucleic acid sequence denoted SEQ ID NO:10. Translation of SEQ ID NO:10 indicates that nucleic acid molecule nhFc.sub..epsilon.R.alpha..su b.612 encodes a ***Fc*** .sub..epsilon.R protein of about 197 amino acids, referred to herein as PhFc.sub..delta.R.alpha..sub.197, having amino acid sequence SEQ ID NO:11. Nucleic acid molecule nhFc.sub..epsilon.R.alpha..sub.612 encodes a secretable form of the ***human*** ***Fc*** .sub..epsilon.R .alpha. chain which does not possess a leader sequence, which is denoted herein as PhFc.sub..epsilon.R.alpha..sub.172 having amino acid sequence SEQ. . . .

DETD [0077] This example describes the biotinylation of a recombinant ***human*** ***Fc*** .sub..epsilon.R alpha chain protein.

DETD [0081] The results shown in FIG. 1 indicate that the alpha chain of ***human*** ***Fc*** .sub..epsilon.R detects the presence of canine IgE (closed circles) in a solid-phase assay in a similar manner as the control antibody. . . .

DETD [0084] The results shown in FIG. 2 indicate that the alpha chain of ***human*** ***Fc*** .sub..epsilon.R detects the presence of canine IgE antibodies that bind specifically to a common grass allergen or to a common tree. . . .

DETD . . . in FIG. 3 indicate that canine IgE from a variety of dog sera are detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R in a manner similar to using an antibody that binds specifically to canine IgE. The absence of detectable amounts of. . . .

DETD . . . 4 indicate that canine IgE that binds specifically to a flea saliva antigen is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R.

DETD . . . IgE from dogs allergic to flea saliva and from dogs infected with heartworm are detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R. In addition, the absence of calorimetric signal in samples of heat inactivated sera indicates that antibody bound to the anti-IgE antibody and detected by ***Fc*** .sub..epsilon.R alpha chain is an epsilon isotype antibody and not another isotype.

DETD . . . indicate that canine IgE that binds specifically to flea saliva, contained in serum, is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R. In addition, the absence of calorimetric signal in samples of heat inactivated serum indicates that antibody bound to the flea saliva protein and detected by ***Fc*** .sub..epsilon.R alpha chain is an epsilon isotype antibody.

DETD . . . feline IgE that binds specifically to crude homogenate of heartworm or Di33 protein is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R.

DETD . . . feline IgE from heartworm-infected cats that specifically binds to the heartworm antigen Di33 is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R. In addition, the absence of calorimetric signal in samples of heat inactivated sera indicates that antibody bound to the Di33 protein and detected by ***Fc*** .sub..epsilon.R alpha chain is an epsilon isotype antibody.

DETD . . . be allergic to certain allergens specifically binds to certain plant and mite allergens is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon.R.

DETD [0106] This example describes detection of canine IgE in a solid-phase ELISA using basophilic cells transfected with ***human*** ***Fc*** .sub..epsilon.R alpha chain.

DETD [0107] Rat basophilic leukemia (RBL) cells transfected with a nucleic acid molecule encoding a ***human*** ***Fc*** .sub..epsilon.R alpha chain (referred to herein as RBL-hFc.sub..epsilon.R cells; described in Miller et al., Science 244:334-337, 1989) were used to detect. . . .

DETD [0110] The results shown in FIG. 10 indicate that canine IgE from

heartworm-infected dogs (.diamond-solid.) is detected using RBL-h ***Fc*** .sub..epsilon.R cells expressing the alpha chain of ***human*** ***Fc*** .sub..epsilon.R. In addition, the absence of calorimetric signal in samples of heat inactivated samples of such sera (.box-solid.) indicates that antibody detected by the ***Fc*** .sub..epsilon.R alpha chain on the RBL-h ***Fc*** .sub..epsilon.R cells is an epsilon isotype antibody. Similarly, the results shown in FIG. 11 indicate that canine IgE from dogs sensitized with flea saliva (.diamond-solid.) is detected using RBL-h ***Fc*** .sub..epsilon.R cells expressing the alpha chain of ***human*** ***Fc*** .sub..epsilon.R. In addition, the absence of calorimetric signal in samples of heat inactivated samples of such sera (.box-solid.) indicates that antibody detected by the ***Fc*** .sub..epsilon.R alpha chain on the RBL-h ***Fc*** .sub..epsilon.R cells is an epsilon isotype antibody.

CLM What is claimed is:

1. A method to detect IgE comprising: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon.R molecule:IgE complex, wherein said IgE is selected from the group consisting of canine IgE, feline IgE and equine IgE; and (b) determining the presence of IgE by detecting said ***Fc*** .sub..epsilon.R molecule:IgE complex, the presence of said ***Fc*** .sub..epsilon.R molecule:IgE complex indicating the presence of IgE.

2. The method of claim 1, wherein said ***Fc*** .sub..epsilon.R molecule comprises at least a portion of a ***Fc*** .sub..epsilon.R alpha chain that binds to IgE.

3. The method of claim 1, wherein said ***Fc*** .sub..epsilon.R molecule comprises a protein selected from the group consisting of PhFc.sub..epsilon.R.alpha..sub.257, PhFc.sub..epsilon.R.alpha..sub.197, PhFc.sub..epsilon.R.alpha..sub.232and PhFc.sub..epsilon.R.alpha..sub.172 .

4. The method of claim 1, wherein said ***Fc*** .sub..epsilon.R molecule is encoded by a nucleic acid molecule selected from the group consisting of nhFc.sub..epsilon.R.alpha..sub.774, nhFc.sub..epsilon.R.alpha..sub.1198, nhFc.sub..epsilon.R.alpha..sub.612, nhFc.sub..epsilon.R.alpha..sub.591, nhFc.sub..epsilon.R.alpha..sub.699 and. . .

5. The method of claim 1, wherein said ***Fc*** .sub..epsilon.R molecule is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising a. . .

6. The method of claim 1, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** .

7. The method of claim 1, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a. . .

8. The method of claim 1, wherein said ***Fc*** R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, biotin-related compounds, avidin, avidin-related compounds and a peroxidase.

. . .

9. The method of claim 1, wherein a carbohydrate group of said ***Fc*** .sub..epsilon.R molecule is conjugated to biotin.

14. The method of claim 1 further comprising the step selected from the group consisting of binding said ***Fc*** .sub..epsilon.R molecule to a substrate prior to performing step (a) to form a ***Fc*** .sub..epsilon.R molecule-coated substrate; and binding said putative IgE-containing composition to a substrate prior to performing step (a) to form a putative IgE-containing composition-coated substrate, wherein said substrate is selected from the group consisting of a non-coated substrate, a ***Fc*** .sub..epsilon.R molecule-coated substrate, an antigen-coated substrate and an anti-IgE antibody-coated substrate.

22. The method of claim 1, wherein said step of detecting comprises: (a) contacting said ***Fc*** .sub..epsilon.R molecule:IgE complex with an indicator molecule that binds selectively to said ***Fc*** .sub..epsilon.R molecule:IgE complex; (b) removing substantially all of said indicator molecule that does not selectively bind to ***Fc*** .sub..epsilon.R molecule:IgE complex; and (c) detecting said indicator molecule, wherein presence of said indicator molecule is indicative of the presence of. . .

. . . . 23. The method of claim 22, wherein said indicator molecule comprises a compound selected from the group consisting of a ***Fc*** .sub..epsilon.R molecule, an antigen, an antibody and a lectin.

24. The method of claim 1, said method comprising the steps of: (a) immobilizing said ***Fc*** .sub..epsilon.R molecule on a substrate; (b) contacting said ***Fc*** .sub..epsilon.R molecule with said putative IgE-containing composition under conditions suitable for formation of an ***Fc*** .sub..epsilon.R molecule:IgE complex bound to said substrate; (c) removing non-bound material from said substrate under conditions that retain ***Fc*** .sub..epsilon.R molecule:IgE complex binding to said substrate; and (d) detecting the presence of said ***Fc*** .sub..epsilon.R molecule:IgE complex.

25. The method of claim 24, wherein the presence of said ***Fc*** .sub..epsilon.R molecule:IgE complex is detected by contacting said ***Fc*** .sub..epsilon.R molecule:IgE complex with a compound selected from the group consisting of an antigen and an antibody that binds selectively to. . .

26. The method of claim 25, wherein said compound comprises a ***detectable*** ***marker*** .

27. The method of claim 1, said method comprising the steps of: (a) immobilizing a desired antigen on a substrate;. . . binding to said substrate; and (d) detecting the presence of said antigen:IgE complex by contacting said antigen:IgE complex with said ***Fc*** .sub..epsilon.R molecule.

28. The method of claim 27, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, avidin, a peroxidase and other members of the. . .

. . . . binding to said substrate; and (d) detecting the presence of said antibody:IgE complex by contacting said antibody:IgE complex with said ***Fc*** .sub..epsilon.R molecule.

30. The method of claim 29, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, a biotin-related compound, avidin, an avidin-related compound and. . .

. . . . method comprising the steps of: (a) immobilizing said putative IgE-containing composition on a substrate; (b) contacting said composition with said ***Fc*** .sub..epsilon.R molecule under conditions suitable for formation of an ***Fc*** .sub..epsilon.R molecule:IgE complex bound to said substrate; (c) removing non-bound material from said substrate under conditions that retain ***Fc*** .sub..epsilon.R molecule:IgE complex binding to said substrate; and (d) detecting the presence of said ***Fc*** .sub..epsilon.R molecule:IgE complex.

32. The method of claim 31, wherein the presence of said ***Fc*** .sub..epsilon.R molecule:IgE complex is detected by contacting said ***Fc*** .sub..epsilon.R molecule:IgE complex with an indicator molecule selected from the group consisting of an antibody, an antigen and a lectin.

33. The method of claim 31, wherein said ***Fc*** .sub..epsilon.R molecule comprises a ***detectable*** ***marker*** .

36. A method to detect IgE comprising: (a) contacting a recombinant cell with a putative IgE-containing composition under conditions

suitable. . . a recombinant cell:IgE complex, wherein said recombinant cell is selected from the group consisting of: a recombinant cell expressing a ***human*** ***Fc*** .sub..epsilon.R molecule; and a recombinant cell expressing an antibody that binds selectively to an IgE selected from the group consisting of. . .
37. The method of claim 36, wherein said recombinant cell expresses a ***Fc*** .sub..epsilon.R molecule comprising at least a portion of a ***human*** ***Fc*** .sub..epsilon.R alpha chain that binds to IgE.

38. The method of claim 36, wherein said recombinant cell expresses a ***Fc*** .sub..epsilon.R molecule comprising a protein selected from the group consisting of PhFc.sub..epsilon.R.alpha..sub.257 and PhFc.sub..epsilon.R.alpha..sub.232.

39. The method of claim 36, wherein said recombinant cell expresses a ***Fc*** .sub..epsilon.R molecule encoded by a nucleic acid molecule selected from the group consisting of nhFc.sub..epsilon.R.alpha..sub.612 , nhFc.sub..epsilon.R.alpha..sub.591, nhFc.sub..epsilon.R.alpha..sub.699 and nhFc.sub..epsilon.R.alpha..sub.516.

40. The method of claim 36, wherein said recombinant cell expresses a ***Fc*** .sub..epsilon.R molecule encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising a nucleic. . .
42. A kit for detecting IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor molecule and a means for detecting an IgE selected from the group consisting of canine IgE, feline IgE and. . .
45. The kit of claim 42, wherein said detection means detects said ***Fc*** R molecule.

46. The kit of claim 42, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to biotin.

47. The kit of claim 42, wherein said ***Fc*** .sub..epsilon.R molecule is on the surface of a recombinant cell that expresses said ***Fc*** .sub..epsilon.R molecule.

54. The kit of claim 42, wherein said ***Fc*** .sub..epsilon.R molecule comprises at least a portion of a ***Fc*** .sub..epsilon.R alpha chain that binds to IgE.

55. The kit of claim 42, wherein said ***Fc*** .sub..epsilon.R molecule comprises a protein selected from the group consisting of PhFc.sub..epsilon.R.alpha..sub.257, PhFc.sub..epsilon.R.alpha..sub.197, PhFc.sub..epsilon.R.alpha..sub.232 and PhFc.sub..epsilon.R.alpha..sub.17 2.

56. The kit of claim 42, wherein said ***Fc*** .sub..epsilon.R molecule is encoded by a nucleic acid molecule selected from the group consisting of nhFc.sub..epsilon.R.alpha..sub.774, nhFc.sub..epsilon.R.alpha..sub.1198, nhFc.sub..epsilon.R.alpha..sub.612, nhFc.sub..epsilon.R.alpha..sub.591, nhFc.sub..epsilon.R.alpha..sub.699 and. . .
57. The kit of claim 42, wherein said ***Fc*** .sub..epsilon.R molecule is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising a. . .
58. The kit of claim 42, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** .

59. The kit of claim 42, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a. . .
60. The kit of claim 42, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, a biotin-related compound, avidin, an avidin-related compound and. . .
61. The kit of claim 42, wherein a carbohydrate group of said ***Fc*** .sub..epsilon.R molecule is conjugated to biotin.

. . . said labeling reagent is impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising said ***Fc*** .sub..epsilon.R molecule, wherein said capture reagent is located downstream of said labeling reagent within a capture zone fluidly connected to said. . .

74. A general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon. receptor molecule.

80. The kit of claim 74, wherein said ***Fc*** .sub..epsilon.R molecule comprises at least a portion of an alpha chain that binds to IgE.

81. The kit of claim 74, wherein said ***Fc*** .sub..epsilon.R molecule comprises a protein selected from the group consisting of PhFc.sub..epsilon.R.alpha..sub.257, PhFc.sub..epsilon.R.alpha..sub.197, PhFc.sub..epsilon.R.alpha..sub.232 and PhFc.sub..epsilon.R.alpha..sub.17 2.

82. The kit of claim 74, wherein said ***Fc*** .sub..epsilon.R molecule is encoded by a nucleic acid molecule selected from the group consisting of nhFc.sub..epsilon.R.alpha..sub.774, nhFc.sub..epsilon.R.alpha..sub.1198, nhFc.sub..epsilon.R.alpha..sub.612, nhFc.sub..epsilon.R.alpha..sub.591, nhFc.sub..epsilon.R.alpha..sub.699 and. . .

83. The kit of claim 74, wherein said ***Fc*** .sub..epsilon.R molecule is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising a. . .

84. The kit of claim 74, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** .

85. The kit of claim 74, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a ligand.

86. The kit of claim 74, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, a biotin-related compound, avidin, an avidin-related compound and. . .

87. The kit of claim 74, wherein a carbohydrate group of said ***Fc*** .sub..epsilon.R molecule is conjugated to biotin.

. . . binding to said substrate; and (d) detecting the presence of said antigen:IgE complex by contacting said antigen:IgE complex with a ***Fc*** .sub..epsilon.R molecule.

93. The method of claim 88, wherein said ***Fc*** .sub..epsilon.R molecule comprises at least a portion of a ***Fc*** .sub..epsilon.R alpha chain that binds to IgE.

94. A kit for detecting flea allergy dermatitis comprising a ***human*** ***Fc*** .sub..epsilon. receptor molecule and a flea allergen.

97. The kit of claim 94, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to a ***detectable*** ***marker*** .

98. The kit of claim 94, wherein said ***Fc*** .sub..epsilon.R molecule is conjugated to biotin.

99. The kit of claim 94, wherein said ***Fc*** .sub..epsilon.R molecule comprises at least a portion of a ***Fc*** .sub..epsilon.R alpha chain that binds to IgE.

100. The kit of claim 99, wherein said ***Fc*** .sub..epsilon.R alpha chain is conjugated to biotin.

101. An isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon.R) alpha chain protein, wherein a carbohydrate group of said ***Fc*** .sub..epsilon.R alpha chain protein is

conjugated to biotin.

102. The ***Fc*** .sub..epsilon.R alpha chain protein of claim 101, wherein said ***Fc*** .sub..epsilon.R alpha chain protein comprises a protein selected from the group consisting of PhFc.sub..epsilon.R.alpha..sub.257, PhFc.sub..epsilon.R.alpha..sub.197, PhFc.sub..epsilon.R.alpha..sub.232 and PhFc.sub..epsilon.R.alpha..sub.172.

103. The ***Fc*** .sub..epsilon.R alpha chain protein of claim 101, wherein said ***Fc*** .sub..epsilon.R alpha chain protein is encoded by a nucleic acid molecule selected from the group consisting of nhFc.sub..epsilon.R.alpha..sub.774, nhFc.sub..epsilon.R.alpha..sub.1198, nhFc.sub..epsilon.R.alpha..sub.612, nhFc.sub..epsilon.R.alpha..sub.591,. . .

104. The ***Fc*** .sub..epsilon.R alpha chain protein of claim 101, wherein said ***Fc*** .sub..epsilon.R alpha chain protein is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule. . .

105. The ***Fc*** .sub..epsilon.R alpha chain protein of claim 101, wherein said ***Fc*** .sub..epsilon.R alpha chain protein comprises PhFc.sub..epsilon.R.alpha..sub.172-BIOT.

L13 ANSWER 13 OF 24 USPATFULL on STN

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TI Morphatides: novel shape and structure libraries

IN Short, Jay M., Rancho Santa Fe, CA, UNITED STATES

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DT Utility

FS APPLICATION

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ECL Exemplary Claim: 1

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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides a method for identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a preselected structure, each complex being designated a morphatide, said method comprising: (a) preparing a library of morphatides, comprised of: (i) a scaffolding component selected from the group consisting of nucleic acid, nucleic acid like molecule or nucleic acid analog having one or more regions of randomized sequence; (ii) one or more linker components; and (iii) one or more agent molecules or type of agent molecules, linked to the scaffolding component by one or more type of linker components; and (b)screening the library of morphatides prepared in step (a) by contacting, binding, or associating the morphatides with one or more suitable target molecules upon which a morphatide performs a preselected or desired function or to which a morphatide binds or associates through a pre-selected structure of said morphatide under conditions permitting said morphatide to perform said preselected or desired function on said target molecules or permitting said morphatide to bind or associate with said target molecules through the preselected structure; (c) separating the morphatides performing the preselected or desired function or binding or associating through the preselected structure, from the library of morphatides and target molecules; thereby identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a pre-selected structure.

SUMM . . . applications are a tribute to its enormous potential. One of the earliest polynucleotides of this type was directed to the ***human*** blood clotting enzyme thrombin (Bock et al., 1992). This

study initiated a search for other thrombin inhibitors based on this. . . al., 1995), as well as against reverse transcriptase of feline immunodeficiency virus (Chen et al., 1996). Other were developed against ***human*** growth factors, such as nerve GF (Binkley et al., 1995), vascular endothelial GF (Jellinek et al., 1994), and basic fibroblast. . .

SUMM . . . al 1996) and D-adensosine (58-mer, Kd=1.7 mM, Klussmann et al. 1996) have been isolated and shown to be stable in ***human*** serum at 37.degree. C. Another example includes chirally pure methylphosphonate linkages that are suitable for generating oligomers capable of efficiently. . .

SUMM [0026] The present invention further provides a morphatide labeled with a ***detectable*** ***marker*** .

DETD . . . a plant, a microbe, an insect, a fish, or a mammal. In a preferred further embodiment the mammal is a ***human*** .

DETD [0129] The present invention provides a morphatide labelled with a ***detectable*** ***marker*** . In an embodiment the ***detectable*** ***marker*** is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label, a chemiluminescent label and. . .

DETD [0169] After PCR amplification the four pools of morphatides are combined. Selection cycles are performed on concanavalin A column immobilized ***human*** thrombin (Bock et al. 1992). The DNA from Example 2 is precipitated and dissolved in selection buffer: (20 mM tris-acetate,. . .

DETD [0233] Binkley, J., Allen, P., Brown, D. M., Green, L. S., Tuerk, G., and Gold, L. (1995). RNA ligands to ***human*** nerve growth factor. Nucl. Acids. Res. 23, 3198-3205.

DETD . . . C., Latham, J. A., Vermaas, E. H., and Toole, J.J. (1992). Selection of single-stranded DNA molecules that bind and inhibit ***human*** thrombin. Nature 355, 564-566.

DETD . . . B. L., Koch, W. M. C., and Gold, L. (1995). Using in vitro selection to direct the covalent attachment of ***human*** immunodeficiency virus type I Rev protein to high affinity RNA ligands. Proc. Natl. Acad. Sci. 92, 12220-12224.

DETD [0271] Kubik, M. F., Stephens, A. W., Schneider, D., Marlar, R. A., and Tasset, D. (1994). High-affinity RNA ligands to ***human*** alpha-thrombin. Nucl. Acids. Res. 22, 2619-2626.

DETD . . . D. J., Feigon, J., Hostomsky, Z., and Gold, L. (1995). High-affinity ssDNA inhibitors of the reverse transcriptase of type I ***human*** immunodeficiency virus. Biochemistry 34, 9599-9610.

DETD . . . E., Rojas, M. E., Leung, L. L. K. and G. S. Gibbs (1995b) Functional mapping of the surface residues of ***human*** thrombin. J. Biol. Chem. 270: 16854-16863

DETD [0297] Tuerk, G., MacDougal, S., and Gold, L. (1992). RNA pseudoknots that inhibit ***human*** immunodeficiency virus type I reverse transcriptase. Proc. Natl. Acad. Sci. 89, 6988-6992.

DETD . . . Williams, P. B., Dreskin, S. C., Jouvin, M. H., Kinet, J. P., and Tasset, D. (1996). High-affinity oligonucleotide ligands to ***human*** IgE inhibit binding to ***Fc*** ***epsilon*** ***receptor*** I. J. Immunol. 157, 221-230.

CLM What is claimed is:

59. The method of claim 58, wherein the mammal is a ***human*** .

68. A morphatide labeled with a ***detectable*** ***marker*** .

69. The morphatide of claim 68, wherein the ***detectable*** ***marker*** is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label, a chemiluminescent label and. . .

L13 ANSWER 14 OF 24 USPATFULL on STN

AN 2002:12241 USPATFULL

TI Morphatides: novel shape and structure libraries

IN Short, Jay M., Encinitas, CA, UNITED STATES

PA INVITROGEN CORPORATION (U.S. corporation)

PI US 2002006620 A1 20020117

AI US 2001-825852 A1 20010403 (9)

RLI Continuation of Ser. No. US 1998-61831, filed on 16 Apr 1998, ABANDONED Continuation-in-part of Ser. No. US 1997-953634, filed on 17 Oct 1997, ABANDONED Continuation-in-part of Ser. No. US 1997-839468, filed on 14

Apr 1997, PENDING
PRAI US 1996-28527P 19961017 (60)
DT Utility
FS APPLICATION
LREP Lisa A. Haile, Ph.D., Gray Cary Ware & Freidenrich LLP, Suite 1600, 4365 Executive Drive, San Diego, CA, 92121-2189
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 7 Drawing Page(s)
LN.CNT 2443
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides a method for identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a preselected structure, each complex being designated a morphatide, said method comprising: (a) preparing a library of morphatides, comprised of: (i) a scaffolding component selected from the group consisting of nucleic acid, nucleic acid like molecule or nucleic acid analog having one or more regions of randomized sequence; (ii) one or more linker components; and (iii) one or more agent molecules or type of agent molecules, linked to the scaffolding component by one or more type of linker components; and (b) screening the library of morphatides prepared in step (a) by contacting, binding, or associating the morphatides with one or more suitable target molecules upon which a morphatide performs a preselected or desired function or to which a morphatide binds or associates through a pre-selected structure of said morphatide under conditions permitting said morphatide to perform said preselected or desired function on said target molecules or permitting said morphatide to bind or associate with said target molecules through the preselected structure; (c) separating the morphatides performing the preselected or desired function or binding or associating through the preselected structure, from the library of morphatides and target molecules; thereby identifying one or more complexes from a library of complexes, wherein said complex or complexes are selected for their ability to perform a preselected or desired function on a target molecule or by having a pre-selected structure.

SUMM . . . applications are a tribute to its enormous potential. One of the earliest polynucleotides of this type was directed to the ***human*** blood clotting enzyme thrombin (Bock et al., 1992). This study initiated a search for other thrombin inhibitors based on this. . . . al., 1995), as well as against reverse transcriptase of feline immunodeficiency virus (Chen et al., 1996). Other were developed against ***human*** growth factors, such as nerve GF (Binkley et al., 1995), vascular endothelial GF (Jellinek et al., 1994), and basic fibroblast. . .

SUMM . . . al 1996) and D-adensosine (58-mer, Kd=1.7 mM, Klussmann et al. 1996) have been isolated and shown to be stable in ***human*** serum at 37.degree. C. Another example includes chirally pure methylphosphonate linkages that are suitable for generating oligomers capable of efficiently. . .

SUMM [0027] The present invention further provides a morphatide labeled with a ***detectable*** ***marker*** .

DETD . . . a plant, a microbe, an insect, a fish, or a mammal. In a preferred further embodiment the mammal is a ***human*** .

DETD [0128] The present invention provides a morphatide labelled with a ***detectable*** ***marker*** . In an embodiment the ***detectable*** ***marker*** is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label, a chemiluminescent label and. . .

DETD [0167] After PCR amplification the four pools of morphatides are combined. Selection cycles are performed on concanavalin A column immobilized ***human*** thrombin (Bock et al. 1992). The DNA from Example 2 is precipitated and dissolved in selection buffer: (20 mM trig-acetate,. . .

DETD [0226] Binkley, J., Allen, P., Brown, D. M., Green, L. S., Tuerk, G., and Gold, L. (1995). RNA ligands to ***human*** nerve growth factor. Nucl. Acids. Res. 23, 3198-3205.

DETD . . . Latham, J. A., Vermaas, E. H., and Toole, J. J. (1992). Selection of single-stranded DNA molecules that bind and inhibit ***human*** thrombin. Nature 355, 564-566.

DETD . . . B. L., Koch, W. M. C., and Gold, L. (1995). Using in vitro selection to direct the covalent attachment of ***human*** immunodeficiency virus type I Rev protein to high affinity RNA ligands. Proc. Natl. Acad. Sci. 92, 12220-12224.

DETD [0265] Kubik, M. F., Stephens, A. W., Schneider, D., Marlar, R. A., and Tasset, D. (1994). High-affinity RNA ligands to ***human*** alphathrombin. Nucl. Acids. Res. 22, 2619-2626.

DETD . . . D. J., Feigon, J., Hostomsky, Z., and Gold, L. (1995). High-affinity ssDNA inhibitors of the reverse transcriptase of type I ***human*** immunodeficiency virus. Biochemistry 34, 9599-9610.

DETD . . . E., Rojas, M. E., Leung, L. L. K. and G. S. Gibbs (1995b) Functional mapping of the surface residues of ***human*** thrombin. J. Biol. Chem. 270: 16854-16863

DETD [0291] Tuerk, G., MacDougal, S., and Gold, L. (1992). RNA pseudoknots that inhibit ***human*** immunodeficiency virus type I reverse transcriptase. Proc. Natl. Acad. Sci. 89, 6988-6992.

DETD . . . Williams, P. B., Dreskin, S. C., Jouvin, M. H., Kinet, J. P., and Tasset, D. (1996). High-affinity oligonucleotide ligands to ***human*** IgE inhibit binding to ***Fc*** ***epsilon*** ***receptor*** I. J. Immunol. 157, 221-230.

L13 ANSWER 15 OF 24 USPATFULL on STN

AN 2002:246848 USPATFULL

TI Dermatophagoides nucleic acid molecules, proteins and uses thereof

IN McCall, Catherine A., Boulder, CO, United States
Hunter, Shirley Wu, Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States

PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)

PI US 6455686 B1 20020924

AI US 1999-292225 19990415 (9)

PRAI US 1998-98909P 19980902 (60)
US 1998-85295P 19980513 (60)
US 1998-98565P 19980417 (60)

DT Utility

FS GRANTED

EXNAM Primary Examiner: Crouch, Deborah; Assistant Examiner: Woitach, Joseph

LREP Heska Corporation

CLMN Number of Claims: 22

ECL Exemplary Claim: 1

DRWN 2 Drawing Figure(s); 2 Drawing Page(s)

LN.CNT 5011

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to high molecular weight Dermatophagoides proteins, nucleic acid molecules encoding such proteins, and therapeutic and diagnostic reagents derived from such proteins.

SUMM . . . of the present invention, nor the relevance of such proteins as being immunoreactive with IgE antibodies in canine, feline, or ***human*** sera.

DETD . . . metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; ***Fc*** receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); a "tag" domain (e.g., at. . .

DETD . . . molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subjected to ***human*** manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the. . .

DETD . . . cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., ***human*** , murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK.sup.31 cells and/or. . .

DETD . . . Particularly preferred animals to test with a skin test of the present invention include humans, canines, felines and equines, with ***human*** , canines and felines being even more preferred. As used herein, canine refers to any member of the dog family, including. . .

DETD . . . having an IgE or IgG isotype. Preferred anti-Der HMW-map antibody to detect include feline antibody, canine antibody, equine antibody and ***human*** antibody, with feline, canine and ***human*** antibody being particularly preferred.

DETD . . . visually (e.g., either by eye or by a machines, such as a

densitometer or spectrophotometer) without the need for a
detectable ***marker*** .

DETD In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the Der HMW-map protein, to antibody bound to the Der HMW-map protein, or to a reagent that selectively binds. . .

DETD . . . a complex is detected by contacting a putative antibody-containing composition with a Der HMW-map protein that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a Der HMW-map protein includes, but is not limited to, a radioactive label, a fluorescent label, an enzyme label, a chemiluminescent label, a chromophoric label or a ligand. A ***detectable*** ***marker*** is conjugated to a Der HMW-map protein in such a manner as not to block the ability of the Der. . .

DETD . . . composition. Preferred lectins include those lectins that bind to high-mannose groups. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

DETD . . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., a ***Fc*** receptor), and an antibody-binding complement protein. Preferred indicator molecules include, but are not limited to, an anti-feline IgE antibody, an anti-feline IgG antibody, an anti-canine IgE antibody, an anti-canine IgG antibody, an anti- ***human*** IgE antibody, and an anti- ***human*** IgG antibody. As used herein, an anti-IgE or anti-IgG antibody includes not only a complete antibody but also any subunit. . .

DETD . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

DETD . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

DETD . . . HMW-map protein and the anti-IgE antibody:IgE complex or anti-IgG antibody:IgG complex. Preferably, the Der HMW-map protein is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess Der HMW-map protein is removed, a developing agent is. . .

DETD . . . complex between the Der HMW-map protein and the IgE or IgG. Preferably, the Der HMW-map protein is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess Der HMW-map protein is removed, a developing agent is. . .

DETD . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . .

DETD . . . antibody capable of selectively binding to an IgE or IgG disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a Der HMW-map protein (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin).

DETD . . . compound include variable regions capable of binding to immune cell specific surface molecules and constant regions capable of binding to ***Fc*** receptors on immune cells, in particular IgE constant regions. Preferred CD8 molecules include at least the extracellular functional domains of. . .

DETD . . . a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, ***human*** serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

DETD . . . that occur when mite allergens contact an animal. For example, IgE that specifically binds to mite allergen becomes coupled with ***Fc*** ***epsilon*** ***receptor*** , resulting in ***Fc***

epsilon ***receptor*** -mediated biological response including release of biological mediators, such as histamine, prostaglandins and/or proteases, that can trigger clinical symptoms of allergy.. . .

DETD . . . regulating adenosine 3',5'-cyclic phosphate (cAMP) activity, and compounds that block IgE activity, such as peptides from IgE or IgE specific ***Fc*** receptors, antibodies specific for peptides from IgE or IgE-specific ***Fc*** receptors, or antibodies capable of blocking binding of IgE to ***Fc*** receptors.

DETD . . . room temperature and then washed four times with PBST. About 100 .mu.l/well of a 1:4000 dilution of 40 .mu.g/ml biotinylated ***human*** ***Fc*** .epsilon.R alpha chain protein (produced as described in Frank et al., WO 98/23964, published Nov. 24, 1997) contained in PBSTFCS was. . .

DETD . . . determined. The plate was incubated for about 1 hour at room temperature and then washed four times with PBST. Biotinylated ***human*** ***Fc*** .epsilon.R alpha chain protein was then added and the presence of IgE bound to the plate was detected using the methods. . .

DETD . . . determined. The plate was incubated for about 1 hour at room temperature and then washed four times with PBST. Biotinylated ***human*** ***Fc*** .epsilon.R alpha chain protein was then added and the presence of IgE bound to the plate was detected using the methods. . .

DETD This example demonstrates the binding of the D. farinae HMW-map composition to ***human*** IgE in ***human*** sera isolated from humans known to be allergic to mite allergens.

DETD A technique called RAST, or radio-allergo-absorbent test, was used because the amount of ***human*** IgE present in ***human*** sera is quite low. RAST was essentially performed as described in Aalberse, RC et al., (1981) J. Allergy Clin Immun.. . . To calculate the unit IU/ml, a standard curve was derived by performing RAST with several dilutions of a well-characterized chimeric ***human*** /mouse IgE monoclonal antibody against Derp2, (***human*** IgE/monoclonal anti-Derp2, following the procedure of Schuurman, et al. (1997) J Allergy Clin Immunol. 99: pp 545-550).

DETD . . . 1, was coupled to 50 mg of CNBr-activated Sepharose 4B (available from Pharmacia, Piscataway, N.J.), according to the manufacturer's protocols. ***Human*** sera were selected (17 different samples, total) on the basis of a positive RAST for whole mite D. farinae extracts,. . .

DETD . . . overnight at 27.degree. C., with shaking. After incubation, the coupled Sepharose was washed five times with PBS-T. Radiolabelled (.sup.125-Iodine) sheep anti- ***human*** IgE, made by standard radioiodination protocols, (diluted in PBS-T with 4.5% bovine serum and 0.5% sheep serum, v/v) in a. . . coupled Sepharose was washed four times with PBS-T and counted in a gamma-counter to determine the amount of radiolabeled sheep anti- ***human*** IgE bound to the HMW-map composition-coupled Sepharose. The results are shown in Table 4.

DETD

TABLE 4

Binding of ***human*** IgE to HMW-map composition from D. farinae

Serum number	RAST, D. farinae whole extract, IU	RAST, HMW-map comps'n., IU

. . .

L13 ANSWER 16 OF 24 USPATFULL on STN

AN 2001:237662 USPATFULL

TI METHOD OF DETECTING AND /OR QUANTIFYING A SPECIFIC IGE ANTIBODY IN A LIGUID SAMPLE

IN NEERVEN, JOOST VAN, Almere, Netherlands

PI US 2001055778 A1 20011227

AI US 1999-467901 A1 19991221 (9)

PRAI DK 1998-1709 19981222
US 1998-113536P 19981222 (60)

DT Utility

FS APPLICATION

LREP FINNEGAN HENDERSON FARABOW, GARRETT & DUNNER L L P, 1300 I STREET N W, WASHINGTON, DC, 200053315

CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN 13 Drawing Page(s)
LN.CNT 822
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of detecting and/or quantifying an IgE antibody specific to a ligand in the form of antigen, antibody or hapten in a liquid sample comprising the steps of

(a) contacting (i) the sample with (ii) a ligand in the form of an antigen, an antibody or a hapten to form a mixture I comprising IgE-containing complexes,

(b) mixing mixture I with a carrier to which is bound (iii) IgE receptor, said IgE receptor being CD23 (***Fc*** .epsilon.RII) and/or ***Fc*** .epsilon.RI, to form a mixture II comprising carrier-bound IgE-containing complexes,

(c) separating the carrier-bound IgE-containing complexes from mixture II, and

(d) determining the amount of the carrier-bound IgE-containing complexes formed.

AB (b) mixing mixture I with a carrier to which is bound (iii) IgE receptor, said IgE receptor being CD23 (***Fc*** .epsilon.RII) and/or ***Fc*** .epsilon.RI, to form a mixture II comprising carrier-bound IgE-containing complexes,

SUMM . . . a method of detecting canine, feline and equine IgE. One embodiment of the method comprises the steps of a) binding ***human*** ***FcE*** receptor (***Fc*** .epsilon.RI) to a substrate, b) contacting the substrate- ***Fc*** .epsilon.RI with an IgE-containing composition to form a complex of substrate- ***Fc*** .epsilon.RI-IgE, c) removing excess non-bound material, d) adding an indicator molecule in the form of e.g. an antigen, which can selectively bind to the IgE of the complex, wherein said indicator molecule may be conjugated to a ***detectable*** ***marker*** , e.g. a fluorescent label or a ligand, such as biotin, e) removing excess indicator molecule and f) measuring the labelled. . .

SUMM . . . generally mentioned that the substrate may be e.g. a particulate material, including magnetic particles, or a recombinant cell expressing the ***Fc*** .epsilon.RI. Also it is generally mentioned that the ***detectable*** ***marker*** may be a chemiluminescent label.

SUMM [0007] The prior art assay disclosed in WO 98/23964 uses an excess of substrate- ***Fc*** .epsilon.RI and hence measures the full content of the specific IgE to be detected as well as other immunoglobulins, e.g. IgG, which may bind to the ***Fc*** .epsilon.RI used. The assay is carried out in strict in vitro conditions involving washing steps after addition of serum to substrate- ***Fc*** .epsilon.RI as well as after addition of antigen.

SUMM . . . IgG antibodies", Bheekha Escura et al., Immunology, Vol. 86, 343-350, 1995, discloses a method comprising the steps of a) incubating mouse/ ***human*** chimeric monoclonal IgE specific to NIP (5-iodo-4-hydroxyl-3-nitrophenacetyl) with allergen-NIP to form a complex, b) incubating the complex with B cells,. . .

SUMM . . . after the priority date of the present application, discloses a method of detecting a biologically active, allergen-specific immunoglobulin using a ***Fc*** ***epsilon*** ***receptor*** I molecule comprising forming a complex of ***Fc*** .epsilon.RI-immunoglobulin-allergen and detecting the complex formed. The document mentions preferred embodiments, wherein allergen conjugated to a plastic bead particle and the. . . sample is contacted to allow for formation of a substrate-bound immunoglobulin-allergen complex, and wherein the said complex is contacted with ***Fc*** .epsilon.RI.

SUMM [0013] (b) mixing mixture I with a carrier to which is bound (iii) IgE receptor, said IgE receptor being CD23 (***Fc*** .epsilon.RII) and/or ***Fc*** .epsilon.RI, to form a mixture II comprising carrier-bound IgE-containing complexes,

SUMM [0016] The low affinity IgE receptor CD23 (***Fc*** .epsilon.RII) is found on the surface of eosinophils, activated B and T cells and dendritic cells. CD23 is a multifunctional receptor,. . .

SUMM [0017] The high affinity IgE receptor ***Fc*** .epsilon.RI is found on the surface of mast cells and basophils, and also on Langerhans cells, monocytes and dendritic cells. ***Fc*** .epsilon.RI has also been shown to play a role in IgE-mediated antigen/allergen presentation (4). IgE may bind to ***Fc*** .epsilon.RI in the form of monomeric IgE, IgE-antigen/allergen and multi-component complexes containing both IgE and antigen/allergen. ***Fc*** .epsilon.RI on mast cells and basophils consists of an .alpha.-chain, a .beta.-chain and a .gamma.-chain, and ***Fc*** .epsilon.RI on Langerhans cells, monocytes and dendritic cells consists of an .alpha.-chain and a .gamma.-chain.

SUMM . . . to measure the level of specific IgE, which in in vivo conditions is able to bind to the CD23 and/or ***Fc*** .epsilon.RI receptors thereby exerting its biological function. In the following, this is referred to as the relevant in vivo level of. . .

SUMM . . . the invention the IgE to be detected is quantified using both CD23 alone to obtain a first measurement and using ***Fc*** .epsilon.RI alone to obtain a second measurement.

SUMM . . . an important role in late phase allergic responses, i.e. in responses appearing between about 6 and 24 hours upon exposure. ***Fc*** .epsilon.RI-triggering of mast cells and basophils after cross-linking of IgE causes the immediate allergic responses. Thus, the biological functions of CD23 and ***Fc*** .epsilon.RI are different, and hence the results obtained with the assay of the invention using as IgE receptor CD23 and ***Fc*** .epsilon.RI, respectively, hold different information about the immunological status of the subject, from which the IgE-containing samples originate. It is therefore advantageous to obtain results for both CD23 and ***Fc*** .epsilon.RI in order to provide a more complete basis for monitoring and evaluating the immunological status of the subject.

SUMM [0047] In another preferred embodiment of the invention a combination of CD23 and ***Fc*** .epsilon.RI is used. In case the carrier used is a particulate material, CD23 and ***Fc*** .epsilon.RI may be bound to separate particles or to the same particles.

SUMM . . . CD23. In other words, it is believed that the complexes of ligand and IgE bind to both CD23 and to ***Fc*** .epsilon.RI, and that the equilibrium is shifted towards binding to CD23 at the ratios of ligand to IgE mentioned above.

SUMM [0056] (b) mixing mixture I with a carrier to which is bound (iii) IgE receptor, said IgE receptor being CD23 (***Fc*** .epsilon.RII) and/or ***Fc*** .epsilon.RI, to form a mixture II comprising carrier-bound IgE-containing complexes,

SUMM . . . of the IgE isotype as well as any other immunoglobulin, which has an affinity for the IgE receptors CD23 and/or ***Fc*** .epsilon.RI.

SUMM [0063] In connection with the present invention the term "IgE receptor" means CD23 (***Fc*** .epsilon.RII) and/or ***Fc*** .epsilon.RI. The term "CD23" means any formulation thereof and any part thereof, including CD23 in pure form or in a mixture,. . . surface of the cell membrane, or a section of the soluble part of the .alpha.-chain may be used as CD23. " ***Fc*** .epsilon.RI" means any formulation thereof and any part thereof, including ***Fc*** .epsilon.RI in pure form or in a mixture, solution or extract; synthetic or recombinant ***Fc*** .epsilon.RI; ***Fc*** .epsilon.RI originating from natural sources; and whole ***Fc*** .epsilon.RI and parts thereof. In particular, only the .alpha.-chain, which is primarily responsible for the binding of IgE, or the soluble. . . outer surface of the cell membrane, or a section of the soluble part of the .alpha.-chain may be used as ***Fc*** .epsilon.RI.

DETD . . . and allergen. In a second step, a particulate carrier to which a number of CD23 molecules (and/or a number of ***Fc*** .epsilon.RI molecules) are bound is added, and the said complexes are bound to the carrier via CD23 to form a mixture. . .

DETD . . . (designated "IgE" in the figure) and a particulate carrier to which a number of CD23 molecules (and/or a number of ***Fc*** .epsilon.RI molecules) are bound, are mixed and incubated to form a mixture II containing carrier-bound complexes including a number of IgE. . .

DETD . . . allergen. In a second incubation step, a particulate carrier to which a number of CD23 molecules (and/or a number of ***Fc*** .epsilon.RI molecules) are bound is added, and the said complexes are

bound to the carrier via CD23 to form a mixture. . .

DETD . . . at 4.degree. C. Subsequently, the cells were washed, and bound complexes were stained by incubating with a biotinylated antibody recognising ***human*** IgE (PU PED0048 from ALK-Abello), followed by wash and incubation with a streptavidin-phycoerythrine conjugate (Southern Biotechnology Associates, sold by KEBO,. . .

DETD [0110] (2) "IgE-antigen complexes enhance ***Fc*** .epsilon.R and Ia expression by murine B lymphocytes", Richards et al., J. Exp. Med., Vol. 168, 571, 1998.

DETD [0112] (4) "The High Affinity IgE Receptor (***Fc*** .epsilon.RI) Mediates IgE-Dependant Allergen Presentationr, Maurer et al., The Journal of Immunology, Vol. 154, 6285-6290, 1995.

CLM What is claimed is:

. . . complexes, (b) mixing mixture I with a carrier to which is bound (iii) IgE receptor, said IgE receptor being CD23 (***Fc*** .epsilon.RII) and/or ***Fc*** .epsilon.RI, to form a mixture II comprising carrier-bound IgE-containing complexes, (c) separating the carrier-bound IgE-containing-complexes from mixture II, and (d) determining. . .

. . . claims, wherein the IgE to be detected is quantified using both CD23 alone to obtain a first measurement and using ***Fc*** .epsilon.RI alone to obtain a second measurement.

. . . thereof, (b) mixing mixture I with a carrier to which is bound (iii) IgE receptor, said IgE receptor being CD23 (***Fc*** .epsilon.RII) and/or ***Fc*** .epsilon.RI, to form a mixture II comprising carrier-bound IgE-containing complexes, (b') separating the carrier-bound IgE-containing complexes from mixture II and washing. . .

L13 ANSWER 17 OF 24 USPATFULL on STN

AN 2001:190910 USPATFULL

TI Method to detect IgE

IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States
Wassom, Donald L., Fort Collins, CO, United States

PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)

PI US 6309832 B1 20011030

AI US 1999-285873 19990331 (9)

RLI Division of Ser. No. US 1996-756387, filed on 26 Nov 1996, now patented, Pat. No. US 5945294

DT Utility

FS GRANTED

EXNAM Primary Examiner: Swartz, Rodney P.

LREP Heska Corporation

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention includes a method to detect IgE using a ***human*** ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon. R) to detect IgE antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

AB The present invention includes a method to detect IgE using a ***human*** ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon. R) to detect IgE antibodies in a biological sample from a cat, a dog, or a horse. The present invention. . .

SUMM Until the discovery of the present invention, detection of IgE in samples obtained from non- ***human*** animals has been hindered by the absence of suitable reagents for detection of IgE. Various compounds have been used to. . . with other antibody idiotypes, such as gamma isotype antibodies. The discovery of the present invention includes the use of a ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon. R) molecule to detect the presence of IgE in a putative IgE-containing composition. A ***Fc*** .sub..epsilon. R molecule provides an advantage over, for example anti-IgE antibodies, to detect IgE because a ***Fc*** .sub..epsilon. R molecule can bind to an IgE with more specificity (i.e., less idiotype cross-reactivity) and more

sensitivity (i.e., affinity) than. . .

SUMM Lowenthal et al., 1993, Annals of Allergy 71:481-484, dog serum can transfer cutaneous reactivity to a ***human*** . While it is possible that Lowenthal et al. properly teach the binding of ***human*** ***Fc*** .sub..epsilon. R to canine IgE. Lowenthal et al., however, do not provide data defining the particular cellular proteins responsible for the. . . by Lowenthal et al. is merely an interpretation. In addition, Lowenthal et al. do not teach the use of purified ***human*** ***Fc*** .sub..epsilon. R to detect canine IgE. The subunits of ***human*** ***Fc*** .sub..epsilon. R have been known as early as 1988 and have never been used to detect canine, feline or equine IgE. Indeed, U.S. Pat. No. 4,962,035, to Leder et al., issued Oct. 9, 1990, discloses ***human*** ***Fc*** .sub..epsilon. R but does not disclose the use of such a ***human*** ***Fc*** .sub..epsilon. R to detect ***human*** or non- ***human*** IgE. The use of purified ***human*** ***Fc*** .sub..epsilon. R avoids complications presented by use of ***Fc*** .sub..epsilon. R bound to a cell, such as non-specific binding of the ***Fc*** .sub..epsilon. R-bearing cell due to additional molecules present on the cell membrane. That purified ***human*** ***Fc*** .sub..epsilon. R detects non- ***human*** IgE is unexpected because inter-species binding between a ***Fc*** .sub..epsilon. R and an IgE is not predictable. For example, ***human*** ***Fc*** .sub..epsilon. R binds to rat IgE but rat ***Fc*** .sub..epsilon. R does not bind to ***human*** IgE.

SUMM The high affinity ***Fc*** .sub..epsilon. R consists of three protein chains, alpha, beta and gamma. Prior investigators have disclosed the nucleic acid sequence for: the. . .

SUMM Thus, methods and kits are needed in the art that will provide specific detection of non- ***human*** IgE.

SUMM . . . that detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon. R molecule:IgE complex, wherein the IgE is selected from the group consisting of canine IgE, feline IgE and equine IgE; and (b) determining the presence of IgE by detecting the ***Fc*** .sub..epsilon. R molecule:IgE complex, the presence of the ***Fc*** .sub..epsilon. R molecule:IgE complex indicating the presence of IgE. A preferred ***Fc*** .sub..epsilon. R molecule in which a carbohydrate group of the ***Fc*** .sub..epsilon. R molecule is conjugated to biotin.

SUMM . . . conditions suitable for formation of a recombinant cell:IgE complex, in which the recombinant cell includes: a recombinant cell expressing a ***human*** ***Fc*** .sub..epsilon. R molecule; and a recombinant cell expressing an antibody that binds selectively to an IgE including canine IgE, feline IgE. . .

SUMM . . . binding to the substrate; and (c) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with a ***Fc*** .sub..epsilon. R molecule. Preferably, the flea allergen is a flea saliva antigen and more preferably flea saliva products and/or flea saliva. . .

SUMM . . . includes a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and a means for detecting an IgE including canine IgE, feline IgE and equine IgE. Another embodiment is a general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule. Another embodiment is a kit for detecting flea allergy dermatitis comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and a flea allergen.

SUMM Another embodiment of the present invention is an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) alpha chain protein, in which a carbohydrate group of the ***Fc*** .sub..epsilon. R alpha chain protein is conjugated to biotin. A preferred ***Fc*** .sub..epsilon. R alpha chain protein comprises PhFc.sub..epsilon. R.alpha..sub.172 --BIOT.

DRWD FIG. 1 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect canine IgE antibodies.

DRWD FIG. 2 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect plant allergen-specific canine IgE antibodies.

DRWD FIG. 3 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect ***human*** or canine IgE antibodies.

DRWD FIG. 4 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect flea allergen-specific canine IgE antibodies.

DRWD FIG. 5 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect flea allergen-specific and heartworm antigen-specific canine IgE antibodies.

DRWD FIG. 6 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect flea saliva-specific canine IgE antibodies.

DRWD FIG. 7 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect heartworm antigen-specific feline IgE antibodies.

DRWD FIG. 8 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect heartworm antigen-specific feline IgE antibodies.

DRWD FIG. 9 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect antigen-specific equine IgE antibodies.

DRWD FIG. 10 depicts ELISA results using basophilic leukemia cells expressing alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect canine IgE antibodies in sera from heartworm-infected dogs.

DRWD FIG. 11 depicts ELISA results using basophilic leukemia cells expressing alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect canine IgE antibodies in sera from flea saliva sensitized dogs.

DETD The present invention relates to the discovery that purified high affinity ***human*** ***Fc*** ***epsilon*** ***receptor*** (i.e., ***Fc*** .sub..epsilon. RI; referred to herein as ***Fc*** .sub..epsilon. R) can be used in certain non- ***human*** (i.e., canine, feline or equine) epsilon immunoglobulin (referred to herein as IgE or IgE antibody)-based detection (e.g., diagnostic, screening) methods and kits. The use of ***human*** ***Fc*** .sub..epsilon. R to detect non- ***human*** IgE is unexpected because canine, feline and equine immune systems are different from the ***human*** immune system, as well as from each other (i.e., molecules important to the immune system usually are species specific).

DETD One embodiment of the present invention is a method to detect a non- ***human*** IgE using an isolated ***human*** ***Fc*** .sub..epsilon. R molecule. It is to be noted that the term "a" entity or "an" entity refers to one or more. . .

DETD According to the present invention, an isolated, or biologically pure, ***Fc*** .sub..epsilon. R molecule, is a molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the molecule has been purified. An isolated ***human*** ***Fc*** .sub..epsilon. R molecule of the present invention can be obtained from its natural source (e.g., from a ***human*** mast cell), can be produced using recombinant DNA technology or can be produced by chemical synthesis.

DETD A ***Fc*** .sub..epsilon. R molecule (also referred to herein as ***Fc*** .sub..epsilon. R or ***Fc*** .sub..epsilon. R protein) of the present invention can be a full-length protein, a portion of a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. A ***Fc*** .sub..epsilon. R molecule of the present invention can comprise a complete ***Fc*** .sub..epsilon. R (i.e., alpha, beta and gamma ***Fc*** .sub..epsilon. R chains), an alpha ***Fc*** .sub..epsilon. R chain (also referred to herein as ***Fc*** .sub..epsilon. R .alpha. chain) or portions thereof. Preferably, a ***Fc*** .sub..epsilon. R molecule comprises at least a portion of a ***Fc*** .sub..epsilon. R .alpha. chain that binds to IgE, i.e., that is capable of forming an immunocomplex with an IgE constant region. Preferably, a ***Fc*** .sub..epsilon. R molecule of the present invention binds to IgE with an affinity of about K.sub.A.apprxeq.10.sup.8, more preferably with an affinity. . .

DETD An isolated ***Fc*** .sub..epsilon. R molecule of the present invention, including a homolog, can be identified in a straight-forward manner by the ***Fc*** .sub..epsilon. R molecule's ability to form an

immunocomplex with an IgE. Examples of ***Fc*** .sub..epsilon. R homologs include ***Fc*** .sub..epsilon. R proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide),. . .

DETD ***Fc*** .sub..epsilon. R homologs can be the result of natural allelic variation or natural mutation. ***Fc*** .sub..epsilon. R homologs of the present invention can also be produced using techniques known in the art including, but not limited. . .

DETD According to the present invention, a ***human*** ***Fc*** .sub..epsilon. R .alpha. chain of the present invention is encoded by at least a portion of the nucleic acid sequence of the coding strand of a cDNA encoding a full-length ***Fc*** .sub..epsilon. R .alpha. chain protein represented herein as SEQ ID NO: 1, the portion at least encoding the IgE binding site of the ***Fc*** .sub..epsilon. R .alpha. chain protein. The double-stranded nucleic acid molecule including both the coding strand having SEQ ID NO: 1 and. . . determined by one skilled in the art and is shown herein as SEQ ID NO:3) is referred to herein as ***Fc*** .sub..epsilon. R nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.1198. Translation of SEQ ID NO: 1 suggests that nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.1198 encodes a full-length ***Fc*** .sub..epsilon. R .alpha. chain protein of about 257 amino acids, referred to herein as PhFc.sub..epsilon. R.alpha..sub.257, represented by SEQ ID NO:2,. . . skilled in the art to make modifications to the respective nucleic acid molecules and proteins to, for example, develop a ***Fc*** .sub..epsilon. R .alpha. chain protein with increased solubility and/or a truncated protein (e.g., a peptide) capable of detecting IgE, e.g., PhFc.sub..epsilon. R.alpha..sub.197 and PhFc.sub..epsilon. R.alpha..sub.172. Preferred ***Fc*** .sub..epsilon. R molecules include PhFc.sub..epsilon. R.alpha..sub.257, PhFc.sub..epsilon. R.alpha..sub.197, PhFc.sub..epsilon.R.alpha..sub.232 and PhFc.sub..epsilon. R.alpha..sub.172. Preferred nucleic acid molecules to encode a ***Fc*** .sub..epsilon. R molecules include nhFc.sub..epsilon. R.alpha..sub.774, nhFc.sub..epsilon. R.alpha..sub.1198, nhFc.sub..epsilon..sub..epsilon. R.alpha..sub.612, nhFc.sub..epsilon. R.alpha..sub.591, nhFc.sub..epsilon. R.alpha..sub.699 and/or nhFc.sub..epsilon. R.alpha..sub.516.

DETD Isolated ***Fc*** .sub..epsilon. R molecule protein of the present invention can be produced by culturing a cell capable of expressing the protein under. . . Suitable and preferred nucleic acid molecules with which to transform a cell are as disclosed herein for suitable and preferred ***Fc*** .sub..epsilon. R nucleic acid molecules per se. Particularly preferred nucleic acid molecules to include in recombinant cells of the present invention. . .

DETD . . . one nucleic acid molecule. Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing a ***Fc*** .sub..epsilon. R molecule protein of the present invention or can be capable of producing such proteins after being transformed with at. . .

DETD . . . . transformed, examples of which are disclosed herein. A particularly preferred recombinant molecule includes pVL-nhFc.sub..epsilon. R.alpha..sub.612. Details regarding the production of ***Fc*** .sub..epsilon. R molecule nucleic acid molecule-containing recombinant molecules are disclosed herein. Particularly preferred recombinant cell of the present invention includes Trichoplusia. . .

DETD A ***Fc*** .sub..epsilon. R molecule of the present invention can include chimeric molecules comprising a portion of a ***Fc*** .sub..epsilon. R molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the ***Fc*** .sub..epsilon. R portion binds to IgE in essentially the same manner as a ***Fc*** .sub..epsilon. R molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of. . .

DETD A ***Fc*** .sub..epsilon. R molecule of the present invention can be contained in a formulation, herein referred to as a ***Fc*** .sub..epsilon. R formulation. For example, a ***Fc*** .sub..epsilon. R can be combined with a buffer in which the ***Fc*** .sub..epsilon. R is solubilized, and/or a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a ***Fc*** .sub..epsilon. R can function to

selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate. . . not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be in mixed with ***Fc*** .sub..epsilon. R or conjugated (i.e., attached) to ***Fc*** .sub..epsilon. R in such a manner as to not substantially interfere with the ability of the ***Fc*** .sub..epsilon. R to selectively bind to IgE.

DETD A ***Fc*** .sub..epsilon. R of the present invention can be bound to the surface of a cell expressing the ***Fc*** .sub..epsilon. R. A preferred ***Fc*** .sub..epsilon. R-bearing cell includes a recombinant cell expressing a nucleic acid molecule encoding a ***human*** ***Fc*** .sub..epsilon. R alpha chain of the present invention. A more preferred recombinant cell of the present invention expresses a nucleic acid. . .

DETD In addition, a ***Fc*** .sub..epsilon. R formulation of the present invention can include not only a ***Fc*** .sub..epsilon. R but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers. . .

DETD The present invention also includes ***human*** ***Fc*** .sub..epsilon. R mimetopes and use thereof to detect IgE. In accordance with the present invention, a "mimetope" refers to any compound that is able to mimic the ability of a ***Fc*** .sub..epsilon. R molecule to bind to IgE. A mimetope can be a peptide that has been modified to decrease its susceptibility. . . by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of ***Fc*** .sub..epsilon. R mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex technology, peptides identified by random screening of peptide libraries and proteins. . .

DETD One embodiment of the present invention is a method to detect non- ***human*** IgE which includes the steps of: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule with a putative IgE-containing composition under conditions suitable for formation of an ***Fc*** .sub..epsilon. R molecule:IgE complex; and (b) detecting levels of IgE by detecting said ***Fc*** .sub..epsilon. R molecule:IgE complex. Presence of such a ***Fc*** .sub..epsilon. R molecule:IgE complex indicates that the animal is producing IgE. Preferred non- ***human*** IgE to detect using a ***human*** ***Fc*** .sub..epsilon. R molecule include canine IgE, feline IgE and equine IgE. The present method can further include the step of determining whether an IgE complexed with a ***Fc*** .sub..epsilon. R molecule is heat labile. Methods to determine heat lability of IgE are disclosed in the Examples section. Preferably, an. . . certain flea or heartworm allergens. Moreover, Applicants believe that identification of heat labile IgE and non-heat labile IgE using a ***human*** ***Fc*** .sub..epsilon. R suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE. As such, a ***Fc*** .sub..epsilon. R molecule of the present invention may be useful for detecting molecules bound by the ***Fc*** .sub..epsilon. R molecule but not identical to a known IgE.

DETD As used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with a ***human*** ***Fc*** .sub..epsilon. R molecule. Formation of a complex between a ***Fc*** .sub..epsilon. R and an IgE refers to the ability of the ***Fc*** .sub..epsilon. R to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a ***Fc*** .sub..epsilon. R of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between a ***Fc*** .sub..epsilon. R and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures,. . .

DETD . . . complexes are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between ***Fc*** .sub..epsilon. R and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in. . .

DETD . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e.,

attachment) of a ***detectable*** ***marker*** to the ***Fc*** .sub..epsilon. R or to a reagent that selectively binds to the ***Fc*** .sub..epsilon. R or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable. . . biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin). Preferably, biotin is conjugated to an alpha chain of a ***Fc*** .sub..epsilon. R. Preferably a carbohydrate group of the ***Fc*** .sub..epsilon. R alpha chain is conjugated to biotin. A preferred ***Fc*** .sub..epsilon. R molecule conjugated to biotin comprises PhFc.sub..epsilon. R.alpha..sub.172 -BIOT (the production of which is described in the Examples section).

DETD In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a ***Fc*** .sub..epsilon. R molecule that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a ***Fc*** .sub..epsilon. R molecule includes, but is not limited to, a radioactive label, a fluorescent label, a chemiluminescent label or a chromophoric label. A ***detectable*** ***marker*** is conjugated to a ***Fc*** .sub..epsilon. R molecule or a reagent in such a manner as not to block the ability of the ***Fc*** .sub..epsilon. R or reagent to bind to the IgE being detected. Preferably, a carbohydrate group of a ***Fc*** .sub..epsilon. R is conjugated to biotin.

DETD In another embodiment, a ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting a putative IgE-containing composition with a ***Fc*** .sub..epsilon. R molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the ***Fc*** .sub..epsilon. R molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, a ***Fc*** .sub..epsilon. R molecule, an antigen, an antibody and a lectin, depending upon which portion of the ***Fc*** .sub..epsilon. R molecule:IgE complex being detected. Preferred identifying labeled compounds that are antibodies include, for example, anti-IgE antibodies and anti- ***Fc*** .sub..epsilon. R antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a ***Fc*** .sub..epsilon. R molecule of the present invention produced in insect cells. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

DETD In one preferred embodiment, a ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to a ***Fc*** .sub..epsilon. R molecule of the present invention. Examples of such a reagent includes, but are not limited to, an antibody that selectively binds to a ***Fc*** .sub..epsilon. R molecule (referred to herein as an anti- ***Fc*** .sub..epsilon. R antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to a ***Fc*** .sub..epsilon. R molecule. ***Fc*** .sub..epsilon. R molecules conjugated to biotin are preferably detected using streptavidin, more preferably using ImmunoPure.RTM. NeutrAvidin (available from Pierce, Rockford, Ill.).

DETD In another preferred embodiment, a ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to an IgE antibody (referred. . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., an ***Fc*** receptor), and an antibody-binding complement protein. Preferred anti-IgE reagents include, but are not limited to, D9, and CMI antibody #9,. . .

DETD . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

DETD A preferred immunoabsorbent assay method includes a step of either: (a) binding an ***Fc*** .sub..epsilon. R molecule to a substrate prior to contacting a ***Fc*** .sub..epsilon. R molecule with a putative IgE-containing composition to form a ***Fc*** .sub..epsilon. R molecule-coated substrate; or (b) binding a putative IgE-containing

composition to a substrate prior to contacting a ***Fc*** .sub..epsilon. R molecule with a putative IgE-containing composition to form a putative IgE-containing composition-coated substrate. Preferably, the substrate includes of a non-coated substrate, a ***Fc*** .sub..epsilon. R molecule-coated substrate, an antigen-coated substrate or an anti-IgE antibody-coated substrate.

DETD . . . upon whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, a ***Fc*** .sub..epsilon. R molecule of the present invention is used as a capture molecule when the ***Fc*** .sub..epsilon. R molecule is bound to a substrate. Alternatively, a ***Fc*** .sub..epsilon. R molecule is used as an indicator molecule when the ***Fc*** .sub..epsilon. R molecule is not bound to a substrate. Suitable molecule for use as capture molecules or indicator molecules include, but are not limited to, a ***Fc*** .sub..epsilon. R molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

DETD . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . . .

DETD . . . molecule that can selectively bind to an IgE bound to the antigen, the indicator molecule can be conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . . . and the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is a ***Fc*** .sub..epsilon. R molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

DETD In one embodiment, a ***Fc*** .sub..epsilon. R molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well. . . . A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for ***Fc*** .sub..epsilon. R molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain ***Fc*** .sub..epsilon. R molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the ***Fc*** .sub..epsilon. R is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the ***Fc*** .sub..epsilon. R molecule:IgE complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . . .

DETD . . . material, if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. A ***Fc*** .sub..epsilon. R molecule is added to the substrate and incubated to allow formation of a complex between the ***Fc*** .sub..epsilon. R molecule and the anti-IgE antibody:IgE complex. Preferably, the ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess ***Fc*** .sub..epsilon. R molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device. . . .

DETD . . . Excess non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A ***Fc*** .sub..epsilon. R molecule is added to the substrate and incubated to allow formation of a complex between the ***Fc*** .sub..epsilon. R molecule and the IgE. Preferably, the ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess ***Fc*** .sub..epsilon. R molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device. . . .

DETD . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a colorimetric marker. Typically, the

labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . . zone which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case a ***Fc*** .sub..epsilon. R molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture reagent. . .

DETD . . . apparatus used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a ***Fc*** .sub..epsilon. R molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a. . .

DETD . . . assay in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to a ***Fc*** .sub..epsilon. R molecule of the present invention and an isolated IgE known to bind to the ***Fc*** .sub..epsilon. R molecule. The absence of binding of the ***Fc*** .sub..epsilon. R molecule to the known IgE indicating the presence of IgE in the putative IgE-containing composition.

DETD . . . detect IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and a means for detecting an IgE including canine IgE, feline IgE and/or equine IgE. Suitable and preferred ***Fc*** .sub..epsilon. R molecules are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the ***Fc*** .sub..epsilon. R molecule or to an IgE. A preferred kit of the present invention further comprises a detection means including one. . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a ***Fc*** .sub..epsilon. R molecule (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including canines, felines and/or equines.

DETD . . . present invention is a general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon. R molecule of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that. . .

DETD . . . such as cod, halibut or and tuna, egg, milk, Brewer's yeast, whole wheat, corn, soybean, cheese and rice, and a ***human*** ***Fc*** .sub..epsilon. R molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

DETD This example describes the construction of a recombinant baculovirus expressing a truncated portion of the a-chain of the ***human*** ***Fc*** .sub..epsilon. receptor.

DETD Recombinant molecule pVL-nhFc.sub..epsilon. R.alpha..sub.612, containing a nucleic acid molecule encoding the extracellular domain of the ***Fc*** .sub..epsilon. R .alpha. chain, operatively linked to baculovirus polyhedron transcription control sequences was produced in the following manner. A cDNA clone encoding the full-length alpha chain (.alpha. chain) of the ***human*** ***Fc*** .sub..epsilon. receptor was obtained from Dr. Jean-Pierre Kinet (Harvard University, Cambridge, Mass.). The cDNA clone included an about 1198 nucleotide insert,. . . SEQ ID NO: 1. Translation of SEQ ID NO: 1 indicates that nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.1198 encodes a full-length ***human*** ***Fc*** .sub..epsilon. receptor .alpha. chain protein of about 257 amino acids, referred to herein as PhFc.sub..epsilon. R.alpha..sub.257, having amino acid sequence SEQ. . . 1. The complement of SEQ ID NO: 1 is represented herein by SEQ ID NO:3. The proposed mature protein (i.e., ***Fc*** .sub..epsilon. R.alpha. chain from which the signal sequence has been cleaved), denoted herein as PhFc.sub..epsilon. R.alpha..sub.232, contains about 232 amino acids. . .

DETD To produce a secreted form of the extracellular domain of the ***Fc*** .sub..epsilon. R .alpha. chain, the hydrophobic transmembrane domain and the cytoplasmic tail of the ***Fc*** .sub..epsilon. R .alpha. chain encoded by nhFc.sub..epsilon. R.alpha..sub.1198 were removed as follows. A ***Fc*** .sub..epsilon. R .alpha. chain extracellular domain nucleic acid molecule-containing fragment of about 612 nucleotides was PCR amplified from nhFc.sub..epsilon. R.alpha..sub.1198 using. . .

produce nhFc.sub..epsilon. R.alpha..sub.612. Nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.612 contained an about 591 nucleotide fragment encoding the extracellular domain of the ***human*** ***Fc*** .sub..epsilon. R .alpha. chain, extending from about nucleotide 107 to about nucleotide 697 of SEQ ID NO 1, denoted herein as. . . denoted SEQ ID NO: 10. Translation of SEQ ID NO: 10 indicates that nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.612 encodes a ***Fc*** .sub..epsilon. R protein of about 197 amino acids, referred to herein as PhFc.sub..epsilon. R.alpha..sub.197, having amino acid sequence SEQ ID NO: 11. Nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.612 encodes a secretable form of the ***human*** ***Fc*** .sub..epsilon. R .alpha. chain which does not possess a leader sequence, which is denoted herein as PhFc.sub..epsilon. R.alpha..sub.172 having amino acid. . .

DETD This example describes the biotinylation of a recombinant ***human*** ***Fc*** .sub..epsilon. R alpha chain protein.

DETD The results shown in FIG. 1 indicate that the alpha chain of ***human*** ***Fc*** .sub..epsilon. R detects the presence of canine IgE (closed circles) in a solid-phase assay in a similar manner as the control. . .

DETD The results shown in FIG. 2 indicate that the alpha chain of ***human*** ***Fc*** .sub..epsilon. R detects the presence of canine IgE antibodies that bind specifically to a common grass allergen or to a common. . .

DETD . . . in FIG. 3 indicate that canine IgE from a variety of dog sera are detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R in a manner similar to using an antibody that binds specifically to canine IgE The absence of detectable amounts. . .

DETD . . . 4 indicate that canine IgE that binds specifically to a flea saliva antigen is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R.

DETD . . . IgE from dogs allergic to flea saliva and from dogs infected with heartworm are detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of colorimetric signal in samples of heat inactivated sera indicates that antibody bound to the anti-IgE antibody and detected by ***Fc*** .sub..epsilon. R alpha chain is an epsilon isotype antibody and not another isotype.

DETD . . . indicate that canine IgE that binds specifically to flea saliva, contained in serum, is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of colorimetric signal in samples of heat inactivated serum indicates that antibody bound to the flea saliva protein and detected by ***Fc*** .sub..epsilon. R alpha chain is an epsilon isotype antibody.

DETD . . . feline IgE that binds specifically to crude homogenate of heartworm or Di33 protein is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R.

DETD . . . feline IgE from heartworm-infected cats that specifically binds to the heartworm antigen Di33 is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of colorimetric signal in samples of heat inactivated sera indicates that antibody bound to the Di33 protein and detected by ***Fc*** .sub..epsilon. R alpha chain is an epsilon isotype antibody.

DETD . . . be allergic to certain allergens specifically binds to certain plant and mite allergens is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R.

DETD This example describes detection of canine IgE in a solid-phase ELISA using basophilic cells transfected with ***human*** ***Fc*** .sub..epsilon. R alpha chain.

DETD Rat basophilic leukemia (RBL) cells transfected with a nucleic acid molecule encoding a ***human*** ***Fc*** .sub..epsilon. R alpha chain (referred to herein as RBL-hFc.sub..epsilon. R cells; described in Miller et al., Science 244:334-337, 1989) were used. . .

DETD The results shown in FIG. 10 indicate that canine IgE from heartworm-infected dogs (.diamond-solid.) is detected using RBL-h ***Fc*** .sub..epsilon. R cells expressing the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of colorimetric signal in samples of heat inactivated samples of such sera (.box-solid.) indicates that antibody detected by the ***Fc*** .sub..epsilon. R alpha chain on the RBL-h ***Fc*** .sub..epsilon. R cells is an epsilon isotype antibody. Similarly, the results shown in FIG. 11 indicate that canine IgE from dogs sensitized with flea saliva

(.diamond-solid.) is detected using RBL-h ***Fc*** .sub..epsilon. R cells expressing the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of colorimetric signal in samples of heat inactivated samples of such sera (.box-solid.) indicates that antibody detected by the ***Fc*** .sub..epsilon. R alpha chain on the RBL-h ***Fc*** .sub..epsilon. R cells is an epsilon isotype antibody.

CLM What is claimed is:

. . . . binding to said substrate; and d. detecting the presence of said antigen:IgE complex by contacting said antigen:IgE complex with a ***Fc*** .sub..epsilon. R molecule and removing non-bound ***Fc*** .sub..epsilon. R molecule under conditions that retain bound ***Fc*** .sub..epsilon. R molecule, the presence of said ***Fc*** .sub..epsilon. R molecule indicating the presence of said IgE:antigen complex.

6. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule comprises at least a portion of a ***Fc*** .sub..epsilon. R alpha chain that binds to IgE.

7. A kit for detecting flea allergy dermatitis comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and at least one flea allergen.

10. The kit of claim 7, wherein said ***human*** ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** .

11. The kit of claim 7, wherein said ***human*** ***Fc*** .sub..epsilon. R molecule is conjugated to biotin.

12. The kit of claim 7, wherein said ***human*** ***Fc*** .sub..epsilon. R molecule comprises at least a portion of a ***Fc*** .sub..epsilon. R alpha chain that binds to IgE.

14. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** .

16. The kit of claim 7, wherein a component selected from the group consisting of said ***human*** ***Fc*** .sub..epsilon. receptor and said at least one flea allergen is immobilized on a substrate.

. . . . said labeling reagent is impregnated within the support structure in a labeling zone; and c. a capture reagent comprising said ***Fc*** .sub..epsilon. R molecule, wherein said capture reagent is located downstream of said labeling reagent within a capture zone fluidly connected to. . .

19. The kit of claim 12, wherein a carbohydrate group of said ***human*** ***Fc*** .sub..epsilon. R alpha chain is conjugated to biotin.

20. A method for the detection of flea allergy dermatitis comprising: a. immobilizing on a substrate an ***Fc*** .sub..epsilon. R molecule; b. contacting said substrate with a putative IgE-containing composition under conditions suitable for formation of complex formation between said IgE and said ***Fc*** .sub..epsilon. R molecule, to form an IgE-containing complex; and c. detecting the presence of said IgE-containing complex by contacting said complex. . .

L13 ANSWER 18 OF 24 USPATFULL on STN

AN 2001:148088 USPATFULL

TI Feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules

IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
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Wassom, Donald L., Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States

PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)

PI US 6284881 B1 20010904

AI US 2000-515431 20000229 (9)

RLI Division of Ser. No. US 1998-5299, filed on 9 Jan 1998, now patented, Pat. No. US 6103494, issued on 15 Aug 2000 Division of Ser. No. US 1996-768964, filed on 19 Dec 1996, now patented, Pat. No. US 5958880, issued on 28 Sep 1999

DT Utility

FS GRANTED

EXNAM Primary Examiner: Gambel, Phillip; Assistant Examiner: Roark, Jessica H.

LREP Heska Corporation

CLMN Number of Claims: 17

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 2360

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, compositions comprising such nucleic acid molecules, and methods to use such nucleic acid molecules to produce feline ***Fc*** ***epsilon*** ***receptor*** alpha chain proteins.

TI Feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules

AB The present invention relates to feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, compositions comprising such nucleic acid molecules, and methods to use such nucleic acid molecules to produce feline ***Fc*** ***epsilon*** ***receptor*** alpha chain proteins.

PARN . . . filed Dec. 19, 1996, and which issued as U.S. Pat. No. 5, 958,880 on Sep. 28, 1999, entitled "NOVEL FELINE ***Fc*** ***EPSILON*** ***RECEPTOR*** ALPHA CHAIN PROTEINS, NUCLEIC ACID MOLECULES AND USES THEREOF", which is incorporated herein by this reference in its entirety.

SUMM The present invention relates to feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of. . .

SUMM Immunological stimulation can be mediated by IgE antibodies when IgE complexes with ***Fc*** ***epsilon*** ***receptors*** . ***Fc*** ***epsilon*** ***receptors*** are found on the surface of certain cell types, such as mast cells. Mast cells store biological mediators including histamine, prostaglandins and proteases. Release of these biological mediators is triggered when IgE antibodies complex with ***Fc*** ***epsilon*** ***receptors*** on the surface of a cell. Clinical symptoms result from the release of the biological mediators into the tissue of. . .

SUMM . . . cross-react with other antibody idiotypes, such as gamma isotype antibodies. Also, creation of reagents capable of inhibiting the activity of ***Fc*** ***epsilon*** ***receptors*** has been limited.

SUMM The discovery of the present invention includes a novel feline ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .epsilon.R.alpha.) protein and the use of such a protein to detect the presence of IgE in a putative IgE-containing composition; to identify inhibitors of biological responses mediated by a feline ***Fc*** .epsilon.R.alpha. protein; and as a therapeutic compound to prevent or treat clinical symptoms that result from feline ***Fc*** .epsilon.R.alpha.-mediated biological responses. When used in an assay to detect IgE, a feline ***Fc*** .epsilon.R.alpha. protein provides an advantage over, for example anti-IgE antibodies, to detect IgE because a feline ***Fc*** .epsilon.R.alpha. protein can bind to an IgE with more specificity (i.e., less idiotype cross-reactivity) and more sensitivity (i.e., affinity) than anti-IgE. . .

SUMM Prior investigators have disclosed the nucleic acid sequence for: the ***human*** ***Fc*** .epsilon.R alpha chain (Kochan et al., Nucleic Acids Res. 16:3584, 1988; Shimizu et al., Proc. Natl. Acad. Sci. USA 85:1907-1911, 1988; and Pang et al., J. Immunol. 151:6166-6174, 1993); the ***human*** ***Fc*** .epsilon.R beta chain (Kuster et al., J. Biol. Chem. 267:12782-12787, 1992); the ***human*** ***Fc*** .epsilon.R gamma chain (Kuster et al., J. Biol. Chem. 265:6448-6452, 1990); and the canine ***Fc*** .epsilon.R alpha chain (GenBank.TM. accession number D16413). Although the subunits of ***human*** ***Fc*** .epsilon.R have been known as early as 1988, they have never been used to identify a feline ***Fc*** .epsilon.R. Similarly, even

though the canine ***Fc*** .epsilon.R chain has been known since 1993, it has never been used to identify a feline ***Fc*** .epsilon.R. Moreover, the determination of ***human*** and canine ***Fc*** ' ***epsilon*** ***receptor*** sequences does not indicate, suggest or predict the cloning of a novel ***Fc*** .epsilon.R.alpha. gene from a different species, in particular, from a feline species.

SUMM . . . processes of the present invention are needed in the art that will provide specific detection of IgE and treatment of ***Fc*** ***epsilon*** ***receptor*** -mediated disease.

SUMM The present invention relates to a novel product and process for detecting IgE and protecting animals from ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. According to the present invention there are provided feline ***Fc*** .epsilon.R.alpha. proteins and mimetopes thereof; feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules, including those that encode such proteins; antibodies raised against such feline ***Fc*** .epsilon.R.alpha. proteins (i.e., anti-feline ***Fc*** .epsilon.R.alpha. antibodies); and other compounds that inhibit the ability of feline ***Fc*** .epsilon.R.alpha. protein to form a complex with IgE (i.e, inhibitory compounds or inhibitors).

SUMM . . . comprising such proteins, mimetopes, nucleic acid molecules, antibodies, andlor inhibitory compounds, as well as use of such therapeutic compositions to ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

SUMM One embodiment of the present invention is an isolated nucleic acid molecule encoding a feline ***Fc*** .epsilon.R.alpha. protein. The feline ***Fc*** .epsilon.R.alpha. protein preferably includes: proteins comprising amino acid sequences SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO: 12 and SEQ. . . allelic variants of a nucleic acid molecules encoding a protein comprising any of the amino acid sequences. Particularly preferred feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules include: nucleic acid molecules comprising nucleic acid sequences SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4,. . .

SUMM The present invention also includes an isolated feline ***Fc*** .epsilon.R.alpha. protein. A preferred feline ***Fc*** .epsilon.R.alpha. protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a nucleic acid sequence including SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:15 and SEQ ID NO:16. Particularly preferred feline ***Fc*** .epsilon.R.alpha. proteins include at least one of the following amino acid sequences: SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12. . .

SUMM The present invention also relates to recombinant molecules, recombinant viruses and recombinant cells that include feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules of the present invention. Also included are methods to produce such nucleic acid molecules, recombinant molecules, recombinant. . .

SUMM . . . detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated feline ***Fc*** .epsilon.R.alpha. molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .epsilon.R.alpha. molecule:IgE complex; and (b) determining the presence of IgE by detecting the ***Fc*** .epsilon.R.alpha. molecule:IgE complex, the presence of the ***Fc*** .epsilon.R.alpha. molecule:IgE complex indicating the presence of IgE. A preferred feline ***Fc*** .epsilon.R.alpha. molecule is one which a carbohydrate group of the feline ***Fc*** .epsilon.R.alpha. molecule is conjugated to biotin.

SUMM . . . IgE-containing composition under conditions suitable for formation of a recombinant cell:IgE complex, in which the recombinant cell comprises a feline ***Fc*** .epsilon.R.alpha. molecule; and (b) determining the presence of IgE by detecting the recombinant cell:IgE complex, the presence of the recombinant cell:IgE complex indicating the presence of IgE. A preferred method to detect IgE comprises: (a) immobilizing the ***Fc*** .epsilon.R.alpha. molecule on a substrate; (b) contacting the ***Fc*** .epsilon.R.alpha. molecule with the putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .epsilon.R.alpha. molecule:IgE complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain ***Fc*** .epsilon.R.alpha. molecule:IgE complex binding to the substrate; and (d) detecting the presence of the ***Fc*** .epsilon.R.alpha. molecule:IgE complex. Another preferred

method to detect IgE comprises: (a) immobilizing a specific antigen on a substrate; (b) contacting the. . . binding to said substrate; and (d) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with said ***Fc*** .epsilon.R.alpha. molecule. Another preferred method to detect IgE comprises: (a) immobilizing an antibody that binds selectively to IgE on a substrate;. . . binding to the substrate; and (d) detecting the presence of the antibody:IgE complex by contacting the antibody:IgE complex with said ***Fc*** .epsilon.R.alpha. molecule. Another preferred method to detect IgE comprises: (a) immobilizing a putative IgE-containing composition on a substrate; (b) contacting the composition with the ***Fc*** .epsilon.R.alpha. molecule under conditions suitable for formation of a ***Fc*** .epsilon.R.alpha. molecule:IgE complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain ***Fc*** .epsilon.R.alpha. molecule:IgE complex binding to the substrate; and (d) detecting the presence of the ***Fc*** .epsilon.R.alpha. molecule:IgE complex

SUMM . . . to the substrate; and (d) detecting the presence of the allergen:IgE complex by contacting said allergen:IgE complex with a feline ***Fc*** .epsilon.R.alpha. protein. Preferably, the flea allergen is a flea saliva antigen and more preferably flea saliva products and/or flea saliva proteins.

SUMM . . . a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a feline ***Fc*** .epsilon.R.alpha. protein and a means for detecting IgE. Another embodiment is a kit for detecting flea allergy dermatitis comprising a feline ***Fc*** .epsilon.R.alpha. protein and a flea allergen.

SUMM The present invention also includes an inhibitor that interferes with formation of a complex between feline ***Fc*** .epsilon.R.alpha. protein and IgE, in which the inhibitor is identified by its ability to interfere with the complex formation. A particularly preferred inhibitor includes a substrate analog of a feline ***Fc*** .epsilon.R.alpha. protein, a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein and a soluble portion of a feline ***Fc*** .epsilon.R.alpha. protein. Also included is a method to identify a compound that interferes with formation of a complex between feline ***Fc*** .epsilon.R.alpha. protein and IgE, the method comprising: (a) contacting an isolated feline ***Fc*** .epsilon.R.alpha. protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the feline ***Fc*** .epsilon.R.alpha. protein forms a complex with IgE; and (b) determining if the putative inhibitory compound inhibits the complex formation. A test kit is also includes to identify a compound capable of interfering with formation of a complex between a feline ***Fc*** .epsilon.R.alpha. protein and IgE, the test kit comprising an isolated feline ***Fc*** .epsilon.R.alpha. protein that can complex with IgE and a means for determining the extent of interference of the complex formation in. . .

SUMM Yet another embodiment of the present invention is a therapeutic composition that is capable of reducing ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. Such a therapeutic composition includes one or more of the following therapeutic compounds: an isolated feline ***Fc*** .epsilon.R.alpha. protein; a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a feline ***Fc*** .epsilon.R.alpha. gene; an isolated antibody that selectively binds to a feline ***Fc*** .epsilon.R.alpha. protein; and an inhibitor that interferes with formation of a complex between a feline ***Fc*** .epsilon.R.alpha. protein and IgE. A method of the present invention includes the step of administering to an animal a therapeutic composition. . .

SUMM Yet another embodiment of the present invention is a method to produce a feline ***Fc*** .epsilon.R.alpha. protein, the method comprising culturing a cell transformed with a nucleic acid molecule encoding a feline ***Fc*** .epsilon.R.alpha. protein.

SUMM The present invention provides for isolated feline ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .epsilon.R.alpha.) proteins, isolated feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules, antibodies directed against feline ***Fc*** .epsilon.R.alpha. proteins and other inhibitors of feline ***Fc*** .epsilon.R.alpha. activity. As used herein, the terms isolated feline ***Fc*** .epsilon.R.alpha. proteins and isolated

feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules refers to feline ***Fc*** .epsilon.R.alpha. proteins and feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules derived from cats and, as such, can be obtained from their natural source or can be produced. . . of the present invention are advantageous because they enable the detection of IgE and the inhibition of IgE or feline ***Fc*** .epsilon.R.alpha. protein activity associated with disease. As used herein, feline ***Fc*** epsilon alpha chain receptor protein can be referred to as ***Fc*** .epsilon.R.alpha. protein or ***Fc*** .epsilon.R.alpha. chain protein.

SUMM One embodiment of the present invention is an isolated protein comprising a feline ***Fc*** .epsilon.R.alpha. protein. It is to be noted that the term "a" or "an" entity refers to one or more of that. . .

SUMM As used herein, an isolated feline ***Fc*** .epsilon.R.alpha. protein can be a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. Preferably, a feline ***Fc*** .epsilon.R.alpha. protein comprises at least a portion of a feline ***Fc*** .epsilon.R.alpha. protein that binds to IgE, i.e., that is capable of forming a complex with an IgE.

SUMM A feline ***Fc*** .epsilon.R.alpha. protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to bind to IgE. Examples of feline ***Fc*** .epsilon.R.alpha. protein homologs include feline ***Fc*** .epsilon.R.alpha. proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted,. . .

SUMM Feline ***Fc*** .epsilon.R.alpha. protein homologs can be the result of natural allelic variation or natural mutation. Feline ***Fc*** .epsilon.R.alpha. protein homologs of the present invention can also be produced using techniques known in the art including, but not limited. . .

SUMM Isolated feline ***Fc*** .epsilon.R.alpha. proteins of the present invention have the further characteristic of being encoded by nucleic acid molecules that hybridize under stringent hybridization conditions to a gene encoding a feline ***Fc*** .epsilon.R.alpha. protein. As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules, including oligonucleotides, are. . .

SUMM As used herein, a feline ***Fc*** .epsilon.R.alpha. gene includes all nucleic acid sequences related to a natural feline ***Fc*** .epsilon.R.alpha. gene such as regulatory regions that control production of the feline ***Fc*** .epsilon.R.alpha. protein encoded by that gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. In one embodiment, a feline ***Fc*** .epsilon.R.alpha. gene of the present invention includes nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID. . .

SUMM . . . other nucleic acid and protein sequences presented herein) represent apparent nucleic acid sequences of certain nucleic acid molecules encoding feline ***Fc*** .epsilon.R.alpha. proteins of the present invention.

SUMM In another embodiment, a feline ***Fc*** .epsilon.R.alpha. gene can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:1, SEQ ID. . . NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:15 and/or SEQ ID NO:16. An allelic variant of a feline ***Fc*** .epsilon.R.alpha. gene is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including. . .

SUMM The minimal size of a ***Fc*** .epsilon.R.alpha. protein homolog of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of. . . length if they are AT-rich. As such, the minimal size of a nucleic acid molecule used to encode a feline ***Fc*** .epsilon.R.alpha. protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of a feline ***Fc*** .epsilon.R.alpha. protein homolog of the present invention is from about 4 to about 6 amino acids in length. There is no. . .

SUMM . . . of the cat family, including domestic cats, wild cats and zoo cats. Examples of cats from which to isolate feline ***Fc*** .epsilon.R.alpha. proteins of the present invention (including isolation of the natural protein or production of the protein by recombinant or synthetic. . .

SUMM Suitable cat cells from which to isolate a feline ***Fc*** .epsilon.R.alpha. protein of the present invention include cells that have ***Fc*** .epsilon.R proteins. Preferred cat cells from which to obtain a feline ***Fc*** .epsilon.R.alpha. protein of the present invention include basophil cells, mast cells, mastocytoma cells, dendritic cells, B lymphocytes, macrophages, eosinophils, and/or monocytes. A feline ***Fc*** .epsilon.R.alpha. of the present invention is preferably obtained from mastocytoma cells, mast cells or basophil cells.

SUMM The present invention also includes mimetopes of feline ***Fc*** .epsilon.R.alpha. proteins of the present invention. As used herein, a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein of the present invention refers to any compound that is able to mimic the activity of such a feline ***Fc*** .epsilon.R.alpha. protein (e.g., ability to bind to IgE), often because the mimetope has a structure that mimics the feline ***Fc*** .epsilon.R.alpha. protein. It is to be noted, however, that the mimetope need not have a structure similar to a feline ***Fc*** .epsilon.R.alpha. protein as long as the mimetope functionally mimics the protein. Mimetopes can be, but are not limited to: peptides that. . . nucleic acids; andlor any other peptidomimetic compounds. Mimetopes of the present invention can be designed using computer-generated structures of feline ***Fc*** .epsilon.R.alpha. proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides. . . other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., a feline IgE ***Fc*** domain or anti-feline ***Fc*** .epsilon.R.alpha. antibody). A mimetope can also be obtained by, for example, rational drug design. In a rational drug design procedure, the. . . for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of feline ***Fc*** .epsilon.R.alpha. mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex.TM. technology, peptides identified by random screening of peptide libraries and proteins identified. . . by phage display technology. A preferred mimetope is a peptidomimetic compound that is structurally and/or functionally similar to a feline ***Fc*** .epsilon.R.alpha. protein of the present invention, particularly to the IgE ***Fc*** domain binding site of the feline ***Fc*** .epsilon.R.alpha. protein. As used herein, the ***Fc*** domain of an antibody refers to the portion of an immunoglobulin that has ***Fc*** receptor binding effector function. Typically, the ***Fc*** domain of an IgE comprises the CH2 and CH3 domains of the heavy chain constant region.

SUMM According to the present invention, a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention refers to: a feline ***Fc*** .epsilon.R.alpha. protein, in particular a soluble feline ***Fc*** .epsilon.R.alpha. protein; a feline ***Fc*** .epsilon.R.alpha. homolog; a feline ***Fc*** .epsilon.R.alpha. mimetope; a feline ***Fc*** .epsilon.R.alpha. substrate analog; or a feline ***Fc*** .epsilon.R.alpha. peptide. Preferably, a feline ***Fc*** .epsilon.R.alpha. molecule binds to IgE.

SUMM One embodiment of a feline ***Fc*** .epsilon.R.alpha. protein of the present invention is a fusion protein that includes a feline ***Fc*** .epsilon.R.alpha. protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but. . . to, segments that can: enhance a protein's stability; act as an immunopotentiator to enhance an immune response against a feline ***Fc*** .epsilon.R.alpha. protein; and/or assist purification of a feline ***Fc*** .epsilon.R.alpha. protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired. . . protein, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the feline ***Fc*** .epsilon.R.alpha.-containing domain of the protein and can be susceptible to cleavage in order to enable straight-forward recovery of a feline ***Fc*** .epsilon.R.alpha. protein. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid molecule that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of a feline ***Fc*** .epsilon.R.alpha.-containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine

segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; ***Fc*** receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); a "tag" domain (e.g., at. . . the domain, such as monoclonal antibodies); and/or a linker and enzyme domain (e.g., alkaline phosphatase domain connected to a feline ***Fc*** .epsilon.R.alpha. protein by a linker). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding. . .

SUMM A preferred feline ***Fc*** .epsilon.R.alpha. protein of the present invention is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with at. . . of the following nucleic acid molecules: nfelFc.sub..epsilon. R.alpha..sub.1069, nfelFc.sub..epsilon. R.alpha..sub.789, nfelFc.sub..epsilon. R.alpha..sub.714, nfelFc.sub..epsilon. R.alpha..sub.597 and nfelFc.sub..epsilon. R.alpha..sub.522. Preferably, the feline ***Fc*** .epsilon.R.alpha. protein binds to IgE. A further preferred isolated protein is encoded by a nucleic acid molecule that hybridizes under stringent. . .

SUMM . . . acid sequences reported in GenBank.TM. indicates that SEQ ID NO:2 showed the most homology, i.e., about 54% identity, with a ***Fc*** ***epsilon*** ***receptor*** alpha chain protein of Homo Sapiens (GenBank accession number J03605).

SUMM More preferred feline ***Fc*** .epsilon.R.alpha. proteins of the present invention include proteins comprising amino acid sequences that are at least about 60%, preferably at least. . .

SUMM More preferred feline ***Fc*** .epsilon.R.alpha. proteins of the present invention include proteins encoded by a nucleic acid molecule comprising at least a portion of nfelFc.sub..epsilon.. . . allelic variants of such nucleic acid molecules, the portion being capable of binding to IgE. More preferred is a feline ***Fc*** .epsilon.R.alpha. protein encoded by nfelFc.sub..epsilon. R.alpha..sub.1069, nfelFc.sub..epsilon. R.alpha..sub.789, nfelFc.sub..epsilon. R.alpha..sub.714, nfelFc.sub..epsilon. R.alpha..sub.597 and nfelFc.sub..epsilon.R.alpha..sub.522, or by an allelic variant of such nucleic acid molecules. Particularly preferred feline ***Fc*** .epsilon.R.alpha. proteins are PfelFc.sub..epsilon. R.alpha..sub.238, PfelFc.sub..epsilon. R.alpha..sub.263, PfelFc.sub..epsilon. R.alpha..sub.199 and PfelFc.sub..epsilon. R.alpha..sub.174.

SUMM In one embodiment, a preferred feline ***Fc*** .epsilon.R.alpha. protein of the present invention is encoded by at least a portion of SEQ ID NO:1, SEQ ID NO:4, SEQ. . .

SUMM Also preferred is a feline ***Fc*** .epsilon.R.alpha. protein encoded by an allelic variant of a nucleic acid molecule comprising at least a portion of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:11 and/or SEQ ID NO:14. Particularly preferred feline ***Fc*** .epsilon.R.alpha. proteins of the present invention include SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12 and SEQ ID NO:13 (including,. . .

SUMM . . . embodiment of the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a feline ***Fc*** .epsilon.R.alpha. gene. The identifying characteristics of such a gene are heretofore described. A nucleic acid molecule of the present invention can include an isolated natural feline ***Fc*** .epsilon.R.alpha. gene or a homolog thereof, the latter of which is described in more detail below. A nucleic acid molecule of. . . . nucleic acid molecule of the present invention is the minimal size that can form a stable hybrid with a feline ***Fc*** .epsilon.R.alpha. gene under stringent hybridization conditions.

SUMM . . . molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to ***human*** manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule of the present invention can be isolated from its natural source or can be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules can include, for example, natural allelic variants and nucleic acid molecules modified by nucleotide insertions, deletions, substitutions,. . . a manner such that the modifications do not substantially interfere with the nucleic

acid molecule's ability to encode a feline ***Fc*** .epsilon.R.alpha. protein of the present invention or to form stable hybrids under stringent conditions with natural gene isolates.

SUMM A feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule homolog can be produced using a number of methods known to those skilled in the art (see,. . . mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with a feline ***Fc*** .epsilon.R.alpha. gene or by screening for function of a protein encoded by the nucleic acid molecule (e.g., ability of a feline ***Fc*** .epsilon.R.alpha. protein to bind IgE).

SUMM . . . isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one feline ***Fc*** .epsilon.R.alpha. protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers. . . interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a feline ***Fc*** .epsilon.R.alpha. protein.

SUMM One embodiment of the present invention is a feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule that hybridizes under stringent hybridization conditions with nucleic acid molecule nfelFc.sub..epsilon. R.alpha..sub.1069 and preferably with a nucleic. . .

SUMM . . . acid sequences reported in GenBank indicates that SEQ ID NO:1 showed the most homology, i.e., about 77% identity a canine ***Fc*** ***epsilon*** ***receptor*** alpha chain gene.

SUMM Preferred feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules include nucleic acid molecules having a nucleic acid sequence that is at least about 80%, preferably at. . .

SUMM . . . ID NO:11, SEQ ID NO:14, SEQ ID NO:15 and/or SEQ ID NO:16, that is capable of hybridizing to a feline ***Fc*** .epsilon.R.alpha. gene of the present invention, as well as allelic variants thereof. A more preferred nucleic acid molecule includes the nucleic. . .

SUMM Knowing the nucleic acid sequences of certain feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of. . . (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules from other cats. Such nucleic acid molecules can be obtained in a variety of ways including screening. . .

SUMM . . . conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising feline ***Fc*** .epsilon.R.alpha. genes or other feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of. . . for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules or therapeutic reagents to inhibit feline ***Fc*** .epsilon.R.alpha. protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also includes the. . .

SUMM . . . is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulation of feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules of the present invention.

SUMM . . . the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed feline ***Fc*** .epsilon.R.alpha. protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences. . .

SUMM . . . that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include nfelFc.sub..epsilon. R.alpha..sub.1069, nfelFc.sub..epsilon.. . .

SUMM . . . production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing feline ***Fc*** .epsilon.R.alpha. proteins of the present invention or can be capable of producing such proteins after being transformed with at least one. . . cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., ***human*** , murine or

chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK.sup.31 cells and/or. . .

SUMM Isolated feline ***Fc*** .epsilon.R.alpha. proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins,. . . permit protein production. An effective medium refers to any medium in which a cell is cultured to produce a feline ***Fc*** .epsilon.R.alpha. protein of the present invention. Such a medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources,. . .

SUMM The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to a feline ***Fc*** .epsilon.R.alpha. protein of the present invention or a mimetope thereof (i.e., anti-feline ***Fc*** .epsilon.R.alpha. antibodies). As used herein, the term "selectively binds to" a feline ***Fc*** .epsilon.R.alpha. protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof. . . in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., ibid. An anti-feline ***Fc*** .epsilon.R.alpha. antibody preferably selectively binds to a feline ***Fc*** .epsilon.R.alpha. protein in such a way as to reduce the activity of that protein.

SUMM . . . antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce feline ***Fc*** .epsilon.R.alpha. proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not. . .

SUMM . . . are within the scope of the present invention. For example, such antibodies can be used (a) as tools to detect ***Fc*** ***epsilon*** ***receptor*** in the presence or absence of IgE and/or (b) as tools to screen expression libraries and/or to recover desired proteins. . . proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target cytotoxic agents to cells having ***Fc*** ***epsilon*** ***receptors*** such as those disclosed herein in order to directly kill such cells. Targeting can be accomplished by conjugating (i.e., stably. . . in the art. Suitable cytotoxic agents are known to those skilled in the art. Antibodies of the present invention, including ***Fc*** .epsilon.R.alpha.-binding portions thereof, can also be used, for example, to inhibit binding of IgE to ***Fc*** ***epsilon*** ***receptors*** , to produce anti-feline ***Fc*** .epsilon.R.alpha. idiotypic antibodies, to purify cells having feline ***Fc*** .epsilon.R.alpha. proteins, to stimulate intracellular signal transduction through a feline ***Fc*** .epsilon.R.alpha. and to identify cells having feline ***Fc*** .epsilon.R.alpha. proteins.

SUMM A feline ***Fc*** .epsilon.R.alpha. molecule of the present invention can include chimeric molecules comprising a portion of a feline ***Fc*** .epsilon.R.alpha. molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the ***Fc*** .epsilon.R.alpha. molecule portion binds to IgE in essentially the same manner as a ***Fc*** .epsilon.R.alpha. molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of an. . .

SUMM A feline ***Fc*** .epsilon.R.alpha. molecule of the present invention can be contained in a formulation, herein referred to as a ***Fc*** .epsilon.R.alpha. molecule formulation. For example, a feline ***Fc*** .epsilon.R.alpha. molecule can be combined with a buffer in which the feline ***Fc*** .epsilon.R.alpha. molecule is solubilized, and/or with a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a feline ***Fc*** .epsilon.R.alpha. molecule can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate. . . not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be mixed with feline ***Fc*** .epsilon.R.alpha. molecules or conjugated (i.e., attached) to feline ***Fc*** .epsilon.R.alpha. molecules in such a manner as to not substantially interfere with the ability of the feline ***Fc*** .epsilon.R.alpha. molecules to selectively bind to IgE.

SUMM A feline ***Fc*** .epsilon.R.alpha. protein of the present invention can be bound to the surface of a cell comprising the feline ***Fc***

.epsilon.R.alpha. protein. A preferred feline ***Fc*** .epsilon.R.alpha. protein-bearing cell includes a recombinant cell comprising a nucleic acid molecule encoding a feline ***Fc*** .epsilon.R.alpha. protein of the present invention. A more preferred recombinant cell of the present invention comprises a nucleic acid molecule that. . . following proteins: PfelFc.sub..epsilon. R.alpha..sub.238 and PfelFc.sub..epsilon. R.alpha..sub.263. An even more preferred recombinant cell comprises a nucleic acid molecule including nfel ***Fc*** .sub..epsilon. R.alpha..sub.1069, nfelFc.sub..epsilon. R.alpha..sub.789 and nfelFc.sub..epsilon. R.alpha..sub.714 with a recombinant cell comprising a nucleic acid molecule comprising a nucleic acid sequence. . .

SUMM In addition, a feline ***Fc*** .epsilon.R.alpha. molecule formulation of the present invention can include not only a feline ***Fc*** .epsilon.R.alpha. molecule but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers. . .

SUMM . . . of the present invention is a method to detect IgE which includes the steps of: (a) contacting an isolated feline ***Fc*** .epsilon.R.alpha. molecule with a putative IgE-containing composition under conditions suitable for formation of a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex; and (b) detecting the presence of IgE by detecting the feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex. Presence of such a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex indicates that the animal is producing IgE. Preferred IgE to detect using a feline ***Fc*** .epsilon.R.alpha. molecule include feline IgE, canine IgE, equine IgE and ***human*** IgE, with feline IgE being particularly preferred. The present method can further include the step of determining whether an IgE complexed with a feline ***Fc*** .epsilon.R.alpha. protein is heat labile. Preferably, a heat labile IgE is determined by incubating an IgE at about 56.degree. C. for. . . or heartworm allergens. Moreover, the inventors believe that identification of heat labile IgE and non-heat labile IgE using a feline ***Fc*** .epsilon.R.alpha. protein suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE antibodies. As such, a feline ***Fc*** .epsilon.R.alpha. protein of the present invention may be useful for detecting molecules bound by the feline ***Fc*** .epsilon.R.alpha. protein but not identical to a known IgE.

SUMM . . . used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with a feline ***Fc*** .epsilon.R.alpha. molecule. Formation of a complex between a feline ***Fc*** .epsilon.R.alpha. molecule and an IgE refers to the ability of the feline ***Fc*** .epsilon.R.alpha. molecule to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between a feline ***Fc*** .epsilon.R.alpha. molecule and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures,. . .

SUMM . . . are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between feline ***Fc*** .epsilon.R.alpha. molecule and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in. . .

SUMM . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the feline ***Fc*** .epsilon.R.alpha. molecule or to a reagent that selectively binds to the feline ***Fc*** .epsilon.R.alpha. molecule or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable. . . compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin available from Pierce, Rockford, Ill.). According to the present invention, a ***detectable*** ***marker*** can be connected to a feline ***Fc*** .epsilon.R.alpha. molecule using, for

example, chemical conjugation or recombinant DNA technology (e.g., connection of a fusion segment such as that described. . . binding domain; an immunoglobulin binding; a sugar binding domain; and a "tag" domain). Preferably a carbohydrate group of the feline ***Fc*** .epsilon.R.alpha. molecule is chemically conjugated to biotin.

SUMM In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a feline ***Fc*** .epsilon.R.alpha. molecule that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a feline ***Fc*** .epsilon.R.alpha. molecule includes, but is not limited to, a radioactive label, a fluorescent label, an enzyme label, a chemiluminescent label, a chromophoric label or a ligand. A ***detectable*** ***marker*** is conjugated to a feline ***Fc*** .epsilon.R.alpha. molecule in such a manner as not to block the ability of the feline ***Fc*** .epsilon.R.alpha. molecule to bind to the IgE being detected. Preferably, a carbohydrate group of a feline ***Fc*** .epsilon.R.alpha. molecule is conjugated to biotin.

SUMM In another embodiment, a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex is detected by contacting a putative IgE-containing composition with a feline ***Fc*** .epsilon.R.alpha. molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the feline ***Fc*** .epsilon.R.alpha. molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, an antigen, an antibody and a lectin, depending upon which portion of the feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex is being detected. Preferred indicator molecules that are antibodies include, for example, anti-IgE antibodies and anti-feline ***Fc*** .epsilon.R.alpha. antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a feline ***Fc*** .epsilon.R.alpha. protein of the present invention produced in insect cells. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

SUMM In one preferred embodiment, a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex is detected by contacting the complex with an indicator molecule that selectively binds to a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention. Examples of such indicator molecule includes, but are not limited to, an antibody that selectively binds to a feline ***Fc*** .epsilon.R.alpha. molecule (referred to herein as an anti-feline ***Fc*** .epsilon.R.alpha. antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to a feline ***Fc*** .epsilon.R.alpha. molecule. A feline ***Fc*** .epsilon.R.alpha. molecule conjugated to biotin is preferably detected using streptavidin, more preferably using ImmunoPure.RTM. NeutrAvidin (available from Pierce, Rockford, Ill.).

SUMM In another preferred embodiment, a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex is detected by contacting the complex with indicator molecule that selectively binds to an IgE antibody (referred to. . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., a ***Fc*** receptor), and an antibody-binding complement protein. A preferred indicator molecule includes an anti-feline IgE antibody. As used herein, an anti-IgE. . .

SUMM . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

SUMM A preferred immunoabsorbent assay method includes a step of either: (a) immobilizing a feline ***Fc*** .epsilon.R.alpha. molecule on a substrate prior to contacting a feline ***Fc*** .epsilon.R.alpha. molecule with a putative IgE-containing composition to form a feline ***Fc*** .epsilon.R.alpha. molecule-immobilized substrate; and (b) binding a putative IgE-containing composition on a substrate prior to contacting a feline ***Fc*** .epsilon.R.alpha. molecule with a putative IgE-containing composition to form a putative IgE-containing composition-bound substrate. Preferably, the substrate includes a non-coated substrate, a feline ***Fc*** .epsilon.R.alpha. molecule-immobilized substrate, an antigen-immobilized substrate or an anti-IgE antibody-immobilized substrate.

SUMM . . . whether the molecule is immobilized to a substrate when the

molecule is exposed to an IgE. For example, a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention is used as a capture molecule when the feline ***Fc*** .epsilon.R.alpha. molecule is bound on a substrate. Alternatively, a feline ***Fc*** .epsilon.R.alpha. molecule is used as an indicator molecule when the feline ***Fc*** .epsilon.R.alpha. molecule is not bound on a substrate. Suitable molecules for use as capture molecules or indicator molecules include, but are not limited to, a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

SUMM . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . . .

SUMM . . . the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is a feline ***Fc*** .epsilon.R.alpha. molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

SUMM In one embodiment, a feline ***Fc*** .epsilon.R.alpha. molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or. . . biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the feline ***Fc*** .epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . .

SUMM . . . if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. A feline ***Fc*** .epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the feline ***Fc*** .epsilon.R.alpha. molecule and the anti-IgE antibody:IgE complex. Preferably, the feline ***Fc*** .epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess feline ***Fc*** .epsilon.R.alpha. molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

SUMM . . . non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A feline ***Fc*** .epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the feline ***Fc*** .epsilon.R.alpha. molecule and the IgE. Preferably, the feline ***Fc*** .epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess feline ***Fc*** .epsilon.R.alpha. molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

SUMM . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a calorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . . which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case a feline ***Fc*** .epsilon.R.alpha. molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture reagent is. . .

SUMM . . . used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a feline ***Fc***

.epsilon.R.alpha. molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a capture. . . .

SUMM . . . in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention and an isolated IgE known to bind to the feline ***Fc*** .epsilon.R.alpha. molecule. The absence of binding of the feline ***Fc*** .epsilon.R.alpha. molecule to the known IgE indicates the presence of IgE in the putative IgE-containing composition. The known IgE is preferably conjugated to a ***detectable*** ***marker*** .

SUMM . . . IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising a feline ***Fc*** .epsilon.R.alpha. protein and a means for detecting an IgE. Suitable and preferred feline ***Fc*** .epsilon.R.alpha. protein are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the feline ***Fc*** .epsilon.R.alpha. protein or to an IgE. A preferred kit of the present invention further comprises a detection means including one or. . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a feline ***Fc*** .epsilon.R.alpha. protein (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including canines, felines and/or equines.

SUMM . . . invention is a general allergen kit comprising an allergen common to all regions of the United States and a feline ***Fc*** .epsilon.R.alpha. protein of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that are. . .

SUMM . . . as cod, halibut or and tuna, egg, milk, Brewer's yeast, whole wheat, corn, soybean, cheese and rice, and a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

SUMM . . . present invention is a therapeutic composition that, when administered to an animal in an effective manner, is capable of reducing ***Fc*** receptor mediated reactions associated with diseases related to biological responses involving ***Fc*** receptor function. A therapeutic composition of the present invention can include: an isolated feline ***Fc*** .epsilon.R.alpha. protein, or homolog thereof; a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a feline ***Fc*** .epsilon.R.alpha. gene; an isolated antibody that selectively binds to a feline ***Fc*** .epsilon.R.alpha. protein; and/or an inhibitor that interferes with formation of a complex between a feline ***Fc*** .epsilon.R.alpha. protein and IgE.

SUMM One embodiment of a therapeutic composition of the present invention is a therapeutic compound comprising a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention, that binds to an IgE. According to the present invention, a feline ***Fc*** .epsilon.R.alpha. molecule competes for IgE with naturally-occurring ***Fc*** ***epsilon*** ***receptors*** , particularly those on mastocytoma cells, mast cells or basophils, so that IgE is bound to the administered feline ***Fc*** .epsilon.R.alpha. molecule and thus is unable to bind to ***Fc*** ***epsilon*** ***receptor*** on a cell, thereby inhibiting mediation of a biological response. Preferred feline ***Fc*** .epsilon.R.alpha. molecule for use in a therapeutic composition comprises a feline ***Fc*** .epsilon.R.alpha. protein, or homolog thereof, as described herein, particularly a fragment thereof, which binds to IgE. Feline ***Fc*** .epsilon.R.alpha. molecules for use in a therapeutic composition can be in a monovalent and/or multivalent form, so long as the feline ***Fc*** .epsilon.R.alpha. molecule is capable of binding to IgE. A more preferred feline ***Fc*** .epsilon.R.alpha. molecule for use in a therapeutic composition includes a soluble fragment of a feline ***Fc*** .epsilon.R.alpha. protein. A preferred feline ***Fc*** .epsilon.R.alpha. protein is encoded by nfelFc.sub..epsilon. R.alpha..sub.522 and an even more preferred feline ***Fc*** .epsilon.R.alpha. protein is PfelFc.sub..epsilon. R.alpha..sub.174.

SUMM . . . therapeutic composition of the present invention comprises a

therapeutic compound that interferes with the formation of a complex between feline ***Fc*** .epsilon.R.alpha. protein and IgE, usually by binding to or otherwise interacting with or otherwise modifying the feline ***Fc*** .epsilon.R.alpha. protein's IgE binding site. Feline ***Fc*** .epsilon.R.alpha. protein inhibitors can also interact with other regions of the feline ***Fc*** .epsilon.R.alpha. protein to inhibit feline ***Fc*** .epsilon.R.alpha. protein activity, for example, by allosteric interaction. An inhibitor of a feline ***Fc*** .epsilon.R.alpha. protein can interfere with ***Fc*** .epsilon.R.alpha. protein and IgE complex formation by, for example, preventing formation of a ***Fc*** .epsilon.R.alpha. protein and IgE complex or disrupting an existing ***Fc*** .epsilon.R.alpha. protein and IgE complex causing the ***Fc*** .epsilon.R.alpha. protein and IgE to dissociate. An inhibitor of a feline ***Fc*** .epsilon.R.alpha. protein is usually a relatively small. Preferably, a feline ***Fc*** .epsilon.R.alpha. protein inhibitor of the present invention is identified by its ability to bind to, or otherwise interact with, a feline ***Fc*** .epsilon.R.alpha. protein, thereby interfering with the formation of a complex between a feline ***Fc*** .epsilon.R.alpha. protein and IgE.

SUMM Preferred inhibitors of a feline ***Fc*** .epsilon.R.alpha. protein of the present invention include, but are not limited to, a substrate analog of a feline ***Fc*** .epsilon.R.alpha. protein, a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein, a soluble (i.e., secreted form of a feline ***Fc*** .epsilon.R.alpha. protein) portion of a feline ***Fc*** .epsilon.R.alpha. protein that binds to IgE, and other molecules that bind to a feline ***Fc*** .epsilon.R.alpha. protein (e.g., to an allosteric site) in such a manner that IgE-binding activity of the feline ***Fc*** .epsilon.R.alpha. protein is inhibited. A feline ***Fc*** .epsilon.R.alpha. protein substrate analog refers to a compound that interacts with (e.g., binds to, associates with, modifies) the IgE-binding site of a feline ***Fc*** .epsilon.R.alpha. protein. A preferred feline ***Fc*** .epsilon.R.alpha. protein substrate analog inhibits IgE-binding activity of a feline ***Fc*** .epsilon.R.alpha. protein. Feline ***Fc*** .epsilon.R.alpha. protein substrate analogs can be of any inorganic or organic composition, and, as such, can be, but are not limited to, peptides, nucleic acids, and peptidomimetic compounds. Feline ***Fc*** .epsilon.R.alpha. protein substrate analogs can be, but need not be, structurally similar to a feline ***Fc*** .epsilon.R.alpha. protein's natural substrate (e.g., IgE) as long as they can interact with the active site (e.g., IgE-binding site of that feline ***Fc*** .epsilon.R.alpha.). Feline ***Fc*** .epsilon.R.alpha. protein substrate analogs can be designed using computer-generated structures of feline ***Fc*** .epsilon.R.alpha. proteins of the present invention or computer structures of, for example, the ***Fc*** domain of IgE. Substrate analogs can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides, peptidomimetic. . . inorganic or organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., a feline ***Fc*** .epsilon.R.alpha. protein or anti-feline ***Fc*** .epsilon.R.alpha. idiotypic antibody). A preferred feline ***Fc*** .epsilon.R.alpha. protein substrate analog is a peptidomimetic compound (i.e., a compound that is structurally and/or functionally similar to a natural substrate of a feline ***Fc*** .epsilon.R.alpha. protein of the present invention, particularly to the region of the substrate that binds to a feline ***Fc*** .epsilon.R.alpha. protein, but that inhibits IgE binding upon interacting with the IgE binding site).

SUMM Feline ***Fc*** .epsilon.R.alpha. molecules, as well as other inhibitors and therapeutic compounds, can be used directly as compounds in compositions of the present. . .

SUMM In one embodiment, a therapeutic composition of the present invention can be used to reduce a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response in an animal by administering such a composition to an animal. Preferably, an animal is treated by administering. . . therapeutic composition of the present invention in such a manner that a therapeutic compound (e.g., an inhibitor of a feline ***Fc*** .epsilon.R.alpha. protein, an anti-feline ***Fc*** .epsilon.R.alpha. antibody, an inhibitor of IgE, or nucleic acid molecules encoding feline ***Fc*** .epsilon.R.alpha. proteins) binds

to an IgE or a ***Fc*** ***epsilon*** ***receptor*** in the animal. Such administration could be by a variety of routes known to those skilled in the art including,. . .

SUMM Compositions of the present invention can be administered to any animal having a ***Fc*** ***epsilon*** ***receptor*** or an IgE that binds to a therapeutic compound of the present invention or to a protein expressed by a. . .

SUMM . . . a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, ***human*** serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

SUMM . . . the blood of an animal at a constant rate sufficient to attain therapeutic dose levels of the composition to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in the animal. As used herein, a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response refers to cellular responses that occur when ***Fc*** ***epsilon*** ***receptor*** is complexed with IgE. For example, a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response includes release of biological mediators, such as histamine, prostaglandins and/or proteases, that can trigger clinical symptoms of allergy.. . .

SUMM . . . of skill in the art in accordance with the given condition of a patient. For example, to regulate an antigen-specific ***Fc*** ***epsilon*** ***receptor*** -mediated response, a therapeutic composition may be administered more frequently when an antigen is present in a patient's environment in high. . .

SUMM . . . can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a feline ***Fc*** .epsilon.R.alpha. protein or a feline ***Fc*** .epsilon.R.alpha. RNA (e.g., antisense RNA, ribozyme, triple helix forms or RNA drug) in the animal. Nucleic acid molecules can be delivered. . .

SUMM . . . the recipient animal and directs the production of a protein or RNA nucleic acid molecule that is capable of reducing ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in the animal. For example, a recombinant virus comprising a feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule of the present invention is administered according to a protocol that results in the animal producing an amount of protein or RNA sufficient to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. A preferred single dose of a recombinant virus of the present invention is from about 1.times.10.sup.4 to about. . .

SUMM . . . a therapeutic composition of the present invention includes recombinant cells of the present invention that comprises at least one feline ***Fc*** .epsilon.R.alpha. of the present invention. Preferred recombinant cells for this embodiment include Salmonella, E. coli, Listeria, Mycobacterium, S. frugiperda, yeast, (including. . .

SUMM . . . an animal an effective amount of a therapeutic composition selected from the group consisting of an inhibitor of a feline ***Fc*** .epsilon.R.alpha. and a feline ***Fc*** .epsilon.R.alpha. protein (including homologs), wherein said feline ***Fc*** .epsilon.R.alpha. is capable of binding to IgE. Suitable therapeutic compositions and methods of administration methods are disclosed herein. According to the. . . invention, a therapeutic composition and method of the present invention can be used to prevent or alleviate symptoms associated with ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

SUMM The efficacy of a therapeutic composition of the present invention to effect ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses can be tested using standard methods for detecting ***Fc*** receptor-mediated immunity including, but not limited to, immediate hypersensitivity, delayed hypersensitivity, antibody-dependent cellular cytotoxicity (ADCC), immune complex activity, mitogenic activity,. . .

SUMM An inhibitor of feline ***Fc*** .epsilon.R.alpha. activity can be identified using feline ***Fc*** .epsilon.R.alpha. proteins of the present invention by determining the ability of an inhibitor to prevent or disrupt complex formation between a feline ***Fc*** .epsilon.R.alpha. protein and IgE. One embodiment of the present invention is a method to identify a compound capable of inhibiting feline ***Fc*** .epsilon.R.alpha. activity. Such a method includes the steps of (a) contacting (e.g., combining, mixing) an isolated feline

Fc .epsilon.R.alpha. protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the feline ***Fc*** .epsilon.R.alpha. protein has IgE binding activity, and (b) determining if the putative inhibitory compound inhibits the IgE binding activity. Putative inhibitory. . .

SUMM The present invention also includes a test kit to identify a compound capable of inhibiting feline ***Fc*** .epsilon.R.alpha. activity. Such a test kit includes: an isolated feline ***Fc*** .epsilon.R.alpha. protein having IgE binding activity or a complex of feline ***Fc*** .epsilon.R.alpha. protein and IgE; and a means for determining the extent of inhibition of IgE binding activity in the presence of. . .

DETD This example describes the isolation, by DNA hybridization, of a nucleic acid molecule encoding a feline ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .epsilon.R.alpha.) protein from Felis domesticus.

DETD A feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule was isolated from a feline (Felis domesticus) mastocytoma cDNA library by hybridizing the library with a mixture of .sup.32 P-labeled cDNA molecules encoding ***human*** and canine ***Fc*** ***epsilon*** ***receptor*** alpha chains, respectively. A feline mastocytoma cDNA library was prepared as follows. Total RNA was extracted from approximately 1.5 grams. . . the feline mastocytoma cDNA library was screened, using duplicate plaque lifts, with a mixture of .sup.32 P-labeled cDNAs encoding the ***human*** ***Fc*** ***epsilon*** ***receptor*** alpha chain (Kochan et al., Nucleic Acids Res., 16:3584, 1988) and the canine ***Fc*** ***epsilon*** ***receptor*** alpha chain (Hayashi et al., GenBank accession number D 16413, 1993), repsectively. A plaque purified clone identified using the above. . .

DETD This Example demonstrates the production of secreted feline ***Fc*** .epsilon.R.alpha. chain protein in eukaryotic cells.

DETD To produce a secreted form of the extracellular domain of the feline ***Fc*** .epsilon.R.alpha. chain, the hydrophobic transmembrane domain and the cytoplasmic tail of the feline ***Fc*** .epsilon.R.alpha. chain encoded by nfelFc.sub..epsilon. R.alpha..sub.1069 were removed as follows. A feline ***Fc*** .epsilon.R.alpha. chain extracellular domain nucleic acid molecule-containing a fragment of about 597 nucleotides was PCR amplified from nfelFc.sub..epsilon. R.alpha..sub.1069 using a. . . pVL-nfelFc.sub..epsilon. R.alpha..sub.597. Nucleic acid molecule Bv-nfelFc.sub..epsilon. R.alpha..sub.597 contained an about 597 nucleotide fragment encoding the extracellular domain of the feline ***Fc*** .epsilon.R.alpha. chain, extending from about nucleotide 65 through about 661 of SEQ ID NO:1, denoted herein as nucleic acid molecule nfelFc.sub..epsilon.. . . acid sequence denoted SEQ ID NO:11. Translation of SEQ ID NO:11 indicates that nucleic acid molecule nfelF.sub..epsilon. R.alpha..sub.597 encodes a ***Fc*** .epsilon.R.alpha. protein of about 199 amino acids, referred to herein as PfelFc.sub..epsilon. R.alpha..sub.199, having amino acid sequence SEQ ID NO:12. Nucleic acid molecule nfelFc.sub..epsilon. R.alpha..sub.597 encodes a secretable form of the feline ***Fc*** .epsilon.R.alpha. chain. The processed protein product encoded by nfelFc.sub..epsilon. R.alpha..sub.597 is about 174 amino acids and does not possess a leader. . .

DETD . . . frugiperda:pVL-nfelFc.sub..epsilon. R.alpha..sub.597. S. frugiperda:pVL-nfelFc.sub..epsilon. R.alpha..sub.597 is cultured using techniques known to those skilled in the art to produce a feline ***Fc*** .epsilon.R.alpha. protein PfelFc.sub..epsilon. R.alpha..sub.199.

CLM What is claimed is:
10. A method to produce a feline ***Fc*** .epsilon.R.alpha. protein, said method comprising culturing a cell transformed with a feline nucleic acid molecule, wherein said feline nucleic acid molecule. . .

L13 ANSWER 19 OF 24 USPATFULL on STN
AN 2000:105679 USPATFULL
TI Feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, and uses thereof
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States

Wassom, Donald L., Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States

PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6103494 20000815
AI US 1998-5299 19980109 (9)
RLI Division of Ser. No. US 1996-768964, filed on 19 Dec 1996, now patented, Pat. No. US 5958880
DT Utility
FS Granted
EXNAM Primary Examiner: Mertz, Prema; Assistant Examiner: Hamud, Fozia
LREP Heska Corporation
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2779
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect IgE using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

TI Feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, and uses thereof

AB The present invention relates to feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of. . . such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

SUMM The present invention relates to feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of. . .

SUMM Immunological stimulation can be mediated by IgE antibodies when IgE complexes with ***Fc*** ***epsilon*** ***receptors*** . ***Fc*** ***epsilon*** ***receptors*** are found on the surface of certain cell types, such as mast cells. Mast cells store biological mediators including histamine, prostaglandins and proteases. Release of these biological mediators is triggered when IgE antibodies complex with ***Fc*** ***epsilon*** ***receptors*** on the surface of a cell. Clinical symptoms result from the release of the biological mediators into the tissue of. . .

SUMM . . . cross-react with other antibody idiotypes, such as gamma isotype antibodies. Also, creation of reagents capable of inhibiting the activity of ***Fc*** ***epsilon*** ***receptors*** has been limited.

SUMM The discovery of the present invention includes a novel feline ***Fc*** ***epsilon*** ***receptor*** alpha chain (***FC*** .epsilon.R.alpha.) protein and the use of such a protein to detect the presence of IgE in a putative IgE-containing composition; to identify inhibitors of biological responses mediated by a feline ***Fc*** .epsilon.R.alpha. protein; and as a therapeutic compound to prevent or treat clinical symptoms that result from feline ***Fc*** .epsilon.R.alpha.-mediated biological responses. When used in an assay to detect IgE, a feline ***Fc*** .epsilon.R.alpha. protein provides an advantage over, for example anti-IgE antibodies, to detect IgE because a feline ***Fc*** .epsilon.R.alpha. protein can bind to an IgE with more specificity (i.e., less idiotype cross-reactivity) and more sensitivity (i.e., affinity) than anti-IgE. . .

SUMM Prior investigators have disclosed the nucleic acid sequence for: the ***human*** ***Fc*** .epsilon.R alpha chain (Kochan et al., Nucleic Acids Res. 16:3584, 1988; Shimizu et al., Proc. Natl. Acad. Sci. USA 85:1907-1911, 1988; and Pang et al., J. Immunol. 151:6166-6174, 1993); the ***human*** ***Fc*** .epsilon.R beta chain (Kuster et al., J. Biol. Chem. 267:12782-12787, 1992); the ***human*** ***Fc***

.epsilon.R gamma chain (Kuster et al., J. Biol. Chem. 265:6448-6452, 1990); and the canine ***Fc*** .epsilon.R alpha chain (GenBank.TM. accession number D16413). Although the subunits of ***human*** ***Fc*** .epsilon.R have been known as early as 1988, they have never been used to identify a feline ***Fc*** .epsilon.R. Similarly, even though the canine ***Fc*** .epsilon.R chain has been known since 1993, it has never been used to identify a feline ***Fc*** .epsilon.R. Moreover, the determination of ***human*** and canine ***Fc*** ***epsilon*** ***receptor*** sequences does not indicate, suggest or predict the cloning of a novel ***Fc*** .epsilon.R.alpha. gene from a different species, in particular, from a feline species.

SUMM . . . processes of the present invention are needed in the art that will provide specific detection of IgE and treatment of ***Fc*** ***epsilon*** ***receptor*** -mediated disease.

SUMM The present invention relates to a novel product and process for detecting IgE and protecting animals from ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. According to the present invention there are provided feline ***Fc*** .epsilon.R.alpha. proteins and mimetopes thereof; feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules, including those that encode such proteins; antibodies raised against such feline ***Fc*** .epsilon.R.alpha. proteins (i.e., anti-feline ***Fc*** .epsilon.R.alpha. antibodies); and other compounds that inhibit the ability of feline ***Fc*** .epsilon.R.alpha. protein to form a complex with IgE (i.e, inhibitory compounds or inhibitors).

SUMM . . . comprising such proteins, mimetopes, nucleic acid molecules, antibodies, and/or inhibitory compounds, as well as use of such therapeutic compositions to ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

SUMM One embodiment of the present invention is an isolated nucleic acid molecule encoding a feline ***Fc*** .epsilon.R.alpha. protein. The feline ***Fc*** .epsilon.R.alpha. protein preferably includes: proteins comprising amino acid sequences SEQ ID) NO:2, SEQ ID NO:7, SEQ ID NO:12 and SEQ ID. . . . allelic variants of a nucleic acid molecules encoding a protein comprising any of the amino acid sequences. Particularly preferred feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules include: nucleic acid molecules comprising nucleic acid sequences SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4,. . . .

SUMM The present invention also includes an isolated feline ***Fc*** .epsilon.R.alpha. protein. A preferred feline ***Fc*** .epsilon.R.alpha. protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a nucleic acid sequence including SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:15 and SEQ ID NO:16. Particularly preferred feline ***Fc*** .epsilon.R.alpha. proteins include at least one of the following amino acid sequences: SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12. . .

SUMM The present invention also relates to recombinant molecules, recombinant viruses and recombinant cells that include feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules of the present invention. Also included are methods to produce such nucleic acid molecules, recombinant molecules, recombinant. . .

SUMM . . . detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated feline ***Fc*** .epsilon.R.alpha. molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .epsilon.R.alpha. molecule:IgE complex; and (b) determining the presence of IgE by detecting the ***Fc*** .epsilon.R.alpha. molecule:IgE complex, the presence of the ***Fc*** .epsilon.R.alpha. molecule:IgE complex indicating the presence of IgE. A preferred feline ***Fc*** .epsilon.R.alpha. molecule is one which a carbohydrate group of the feline ***Fc*** .epsilon.R.alpha. molecule is conjugated to biotin.

SUMM . . . IgE-containing composition under conditions suitable for formation of a recombinant cell:IgE complex, in which the recombinant cell comprises a feline ***Fc*** .epsilon.R.alpha. molecule; and (b) determining the presence of IgE by detecting the recombinant cell:IgE complex, the presence of the recombinant cell:IgE complex indicating the presence of IgE. A preferred method to detect IgE comprises: (a) immobilizing the ***Fc*** .epsilon.R.alpha. molecule on a substrate; (b) contacting the ***Fc*** .epsilon.R.alpha. molecule with the putative IgE-containing composition under conditions suitable for

formation of a ***Fc*** .epsilon.R.alpha. molecule:IgE complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain ***Fc*** .epsilon.R.alpha. molecule:IgE complex binding to the substrate; and (d) detecting the presence of the ***Fc*** .epsilon.R.alpha. molecule:IgE complex. Another preferred method to detect IgE comprises: (a) immobilizing a specific antigen on a substrate; (b) contacting the. . . binding to said substrate; and (d) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with said ***Fc*** .epsilon.R.alpha. molecule. Another preferred method to detect IgE comprises: (a) immobilizing an antibody that binds selectively to IgE on a substrate;. . . binding to the substrate; and (d) detecting the presence of the antibody:IgE complex by contacting the antibody:IgE complex with said ***Fc*** .epsilon.R.alpha. molecule. Another preferred method to detect IgE comprises: (a) immobilizing a putative IgE-containing composition on a substrate; (b) contacting the composition with the ***Fc*** .epsilon.R.alpha. molecule under conditions suitable for formation of a ***Fc*** .epsilon.R.alpha. molecule:IgE complex bound to the substrate; (c) removing non- bound material from the substrate under conditions that retain ***Fc*** .epsilon.R.alpha. molecule:IgE complex binding to the substrate; and (d) detecting the presence of the ***Fc*** .epsilon.R.alpha. molecule:IgE complex.

SUMM . . . to the substrate; and (d) detecting the presence of the allergen:IgE complex by contacting said allergen:IgE complex with a feline ***Fc*** .epsilon.R.alpha. protein. Preferably, the flea allergen is a flea saliva antigen and more preferably flea saliva products and/or flea saliva proteins.

SUMM . . . a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a feline ***Fc*** .epsilon.R.alpha. protein and a means for detecting IgE. Another embodiment is a kit for detecting flea allergy dermatitis comprising a feline ***Fc*** .epsilon.R.alpha. protein and a flea allergen.

SUMM The present invention also includes an inhibitor that interferes with formation of a complex between feline ***Fc*** .epsilon.R.alpha. protein and IgE, in which the inhibitor is identified by its ability to interfere with the complex formation. A particularly preferred inhibitor includes a substrate analog of a feline ***Fc*** .epsilon.R.alpha. protein, a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein and a soluble portion of a feline ***Fc*** .epsilon.R.alpha. protein. Also included is a method to identify a compound that interferes with formation of a complex between feline ***Fc*** .epsilon.R.alpha. protein and IgE, the method comprising: (a) contacting an isolated feline ***Fc*** .epsilon.R.alpha. protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the feline ***Fc*** .epsilon.R.alpha. protein forms a complex with IgE; and (b) determining if the putative inhibitory compound inhibits the complex formation. A test kit is also includes to identify a compound capable of interfering with formation of a complex between a feline ***Fc*** .epsilon.R.alpha. protein and IgE, the test kit comprising an isolated feline ***Fc*** .epsilon.R.alpha. protein that can complex with IgE and a means for determining the extent of interference of the complex formation in. . .

SUMM Yet another embodiment of the present invention is a therapeutic composition that is capable of reducing ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. Such a therapeutic composition includes one or more of the following therapeutic compounds: an isolated feline ***Fc*** .epsilon.R.alpha. protein; a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a feline ***Fc*** .epsilon.R.alpha. gene; an isolated antibody that selectively binds to a feline ***Fc*** .epsilon.R.alpha. protein; and an inhibitor that interferes with formation of a complex between a feline ***Fc*** .epsilon.R.alpha. protein and IgE. A method of the present invention includes the step of administering to an animal a therapeutic composition. . .

SUMM Yet another embodiment of the present invention is a method to produce a feline ***Fc*** .epsilon.R.alpha. protein, the method comprising culturing a cell transformed with a nucleic acid molecule encoding a feline ***Fc*** .epsilon.R.alpha. protein.

SUMM The present invention provides for isolated feline ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc***

.epsilon.R.alpha.) proteins, isolated feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules, antibodies directed against feline ***Fc*** .epsilon.R.alpha. proteins and other inhibitors of feline ***Fc*** .epsilon.R.alpha. activity. As used herein, the terms isolated feline ***Fc*** .epsilon.R.alpha. proteins and isolated feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules refers to feline ***Fc*** .epsilon.R.alpha. proteins and feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules derived from cats and, as such, can be obtained from their natural source or can be produced. . . of the present invention are advantageous because they enable the detection of IgE and the inhibition of IgE or feline ***Fc*** .epsilon.R.alpha. protein activity associated with disease. As used herein, feline ***Fc*** epsilon alpha chain receptor protein can be referred to as ***Fc*** .epsilon.R.alpha. protein or ***Fc*** .epsilon.R.alpha. chain protein.

SUMM One embodiment of the present invention is an isolated protein comprising a feline ***Fc*** .epsilon.R.alpha. protein. It is to be noted that the term "a" or "an" entity refers to one or more of that. . .

SUMM As used herein, an isolated feline ***Fc*** .epsilon.R.alpha. protein can be a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. Preferably, a feline ***Fc*** .epsilon.R.alpha. protein comprises at least a portion of a feline ***Fc*** .epsilon.R.alpha. protein that binds to IgE, i.e., that is capable of forming a complex with an IgE.

SUMM A feline ***Fc*** .epsilon.R.alpha. protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to bind to IgE. Examples of feline ***Fc*** .epsilon.R.alpha. protein homologs include feline ***Fc*** .epsilon.R.alpha. proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted,. . .

SUMM Feline ***Fc*** .epsilon.R.alpha. protein homologs can be the result of natural allelic variation or natural mutation. Feline ***Fc*** .epsilon.R.alpha. protein homologs of the present invention can also be produced using techniques known in the art including, but not limited. . .

SUMM Isolated feline ***Fc*** .epsilon.R.alpha. proteins of the present invention have the further characteristic of being encoded by nucleic acid molecules that hybridize under stringent hybridization conditions to a gene encoding a feline ***Fc*** .epsilon.R.alpha. protein. As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules, including oligonucleotides, are. . .

SUMM As used herein, a feline ***Fc*** .epsilon.R.alpha. gene includes all nucleic acid sequences related to a natural feline ***Fc*** .epsilon.R.alpha. gene such as regulatory regions that control production of the feline ***Fc*** .epsilon.R.alpha. protein encoded by that gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. In one embodiment, a feline ***Fc*** .epsilon.R.alpha. gene of the present invention includes nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID. . .

SUMM . . . other nucleic acid and protein sequences presented herein). represent apparent nucleic acid sequences of certain nucleic acid molecules encoding feline ***Fc*** .epsilon.R.alpha. proteins of the present invention.

SUMM In another embodiment, a feline ***Fc*** .epsilon.R.alpha. gene can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:1, SEQ ID. . . NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:15 and/or SEQ ID NO:16. An allelic variant of a feline ***Fc*** .epsilon.R.alpha. gene is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including. . .

SUMM The minimal size of a ***Fc*** .epsilon.R.alpha. protein homolog of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of. . . length if they are AT-rich. As such, the minimal size of a nucleic acid molecule used to encode a feline ***Fc*** .epsilon.R.alpha. protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of a feline ***Fc*** .epsilon.R.alpha. protein homolog of the present invention is from about 4 to about 6 amino acids in length. There is no. . .

SUMM . . . of the cat family, including domestic cats, wild cats and zoo cats. Examples of cats from which to isolate feline ***Fc*** .epsilon.R.alpha. proteins of the present invention (including isolation of the natural protein or production of the protein by recombinant or synthetic. . .

SUMM Suitable cat cells from which to isolate a feline ***Fc*** .epsilon.R.alpha. protein of the present invention include cells that have ***Fc*** .epsilon.R.alpha. proteins. Preferred cat cells from which to obtain a feline ***Fc*** .epsilon.R.alpha. protein of the present invention include basophil cells, mast cells, mastocytoma cells, dendritic cells, B lymphocytes, macrophages, eosinophils, and/or monocytes. A feline ***Fc*** .epsilon.R.alpha. of the present invention is preferably obtained from mastocytoma cells, mast cells or basophil cells.

SUMM The present invention also includes mimetopes of feline ***Fc*** .epsilon.R.alpha. proteins of the present invention. As used herein, a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein of the present invention refers to any compound that is able to mimic the activity of such a feline ***Fc*** .epsilon.R.alpha. protein (e.g., ability to bind to IgE), often because the mimetope has a structure that mimics the feline ***Fc*** .epsilon.R.alpha. protein. It is to be noted, however, that the mimetope need not have a structure similar to a feline ***Fc*** .epsilon.R.alpha. protein as long as the mimetope functionally mimics the protein. Mimetopes can be, but are not limited to: peptides that. . . nucleic acids; and/or any other peptidomimetic compounds. Mimetopes of the present invention can be designed using computer-generated structures of feline ***Fc*** .epsilon.R.alpha. proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides. . . other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., a feline IgE ***Fc*** domain or anti-feline ***Fc*** .epsilon.R.alpha. antibody). A mimetope can also be obtained by, for example, rational drug design. In a rational drug design procedure, the. . . for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of feline ***Fc*** .epsilon.R.alpha. mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex.TM. technology, peptides identified by random screening of peptide libraries and proteins identified. . . by phage display technology. A preferred mimetope is a peptidomimetic compound that is structurally and/or functionally similar to a feline ***Fc*** .epsilon.R.alpha. protein of the present invention, particularly to the IgE ***Fc*** domain binding site of the feline ***Fc*** .epsilon.R.alpha. protein. As used herein, the ***Fc*** domain of an antibody refers to the portion of an immunoglobulin that has ***Fc*** receptor binding effector function. Typically, the ***Fc*** domain of an IgE comprises the CH2 and CH3 domains of the heavy chain constant region.

SUMM According to the present invention, a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention refers to: a feline ***Fc*** .epsilon.R.alpha. protein, in particular a soluble feline ***Fc*** .epsilon.R.alpha. protein; a feline ***Fc*** .epsilon.R.alpha. homolog; a feline ***Fc*** .epsilon.R.alpha. mimetope; a feline ***Fc*** .epsilon.R.alpha. substrate analog; or a feline ***Fc*** .epsilon.R.alpha. peptide. Preferably, a feline ***Fc*** .epsilon.R.alpha. molecule binds to IgE.

SUMM One embodiment of a feline ***Fc*** .epsilon.R.alpha. protein of the present invention is a fusion protein that includes a feline ***Fc*** .epsilon.R.alpha. protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but. . . to, segments that can: enhance a protein's stability; act as an immunopotentiator to enhance an immune response against a feline ***Fc*** .epsilon.R.alpha. protein; and/or assist purification of a feline ***Fc*** .epsilon.R.alpha. protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired. . . protein, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the feline ***Fc*** .epsilon.R.alpha.-containing domain of the protein and can be susceptible to cleavage in order to enable straight-forward recovery of a feline ***Fc*** .epsilon.R.alpha. protein. Fusion proteins are

preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid molecule that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of a feline ***Fc*** .epsilon.R.alpha.-containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; ***Fc*** receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); a "tag" domain (e.g., at. . . the domain, such as monoclonal antibodies); and/or a linker and enzyme domain (e.g., alkaline phosphatase domain connected to a feline ***Fc*** .epsilon.R.alpha. protein by a linker). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding. . .

SUMM A preferred feline ***Fc*** .epsilon.R.alpha. protein of the present invention is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with at. . . of the following nucleic acid molecules: nfelFc.sub..epsilon. R.alpha..sub.1069, nfelFc.sub..epsilon. R.alpha..sub.789, nfelFc.sub..epsilon. R.alpha..sub.714, nfelFc.sub..epsilon. R.alpha..sub.597 and nfelFc.sub..epsilon. R.alpha..sub.522. Preferably, the feline ***Fc*** .epsilon.R.alpha. protein binds to IgE. A further preferred isolated protein is encoded by a nucleic acid molecule that hybridizes under stringent. . .

SUMM . . . acid sequences reported in GenBank.TM. indicates that SEQ ID NO:2 showed the most homology, i.e., about 54% identity, with a ***Fc*** ***epsilon*** ***receptor*** alpha chain protein of Homo Sapiens (GenBank accession number J03605).

SUMM More preferred feline ***Fc*** .epsilon.R.alpha. proteins of the present invention include proteins comprising amino acid sequences that are at least about 60%, preferably at least. . .

SUMM More preferred feline ***Fc*** .epsilon.R.alpha. proteins of the present invention include proteins encoded by a nucleic acid molecule comprising at least a portion of nfelFc.sub..epsilon.. . . allelic variants of such nucleic acid molecules, the portion being capable of binding to IgE. More preferred is a feline ***Fc*** .epsilon.R.alpha. protein encoded by nfelFc.sub..epsilon. R.alpha..sub.1069, nfelFc.sub..epsilon. R.alpha..sub.789, nfelFc.sub..epsilon. R.alpha..sub.714, nfelFc.sub..epsilon. R.alpha..sub.597 and nfelFc.sub..epsilon. R.alpha..sub.522, or by an allelic variant of such nucleic acid molecules. Particularly preferred feline ***Fc*** .epsilon.R.alpha. proteins are PfelFc.sub..epsilon. R.alpha..sub.238, PfelFc.sub..epsilon. R.alpha..sub.263, PfelFc.sub..epsilon. R.alpha..sub.199 and PfelFc.sub..epsilon. R.alpha..sub.174.

SUMM In one embodiment, a preferred feline ***Fc*** .epsilon.R.alpha. protein of the present invention is encoded by at least a portion of SEQ ID NO:1, SEQ ID NO:4, SEQ. . .

SUMM Also preferred is a feline ***Fc*** .epsilon.R.alpha. protein encoded by an allelic variant of a nucleic acid molecule comprising at least a portion of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:11 and/or SEQ ID NO:14. Particularly preferred feline ***Fc*** .epsilon.R.alpha. proteins of the present invention include SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12 and SEQ ID NO:13 (including,. . .

SUMM . . . embodiment of the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a feline ***Fc*** .epsilon.R.alpha. gene. The identifying characteristics of such a gene are heretofore described. A nucleic acid molecule of the present invention can include an isolated natural feline ***Fc*** .epsilon.R.alpha. gene or a homolog thereof, the latter of which is described in more detail below. A nucleic acid molecule of. . . nucleic acid molecule of the present invention is the minimal size that can form a stable hybrid with a feline ***Fc*** .epsilon.R.alpha. gene under stringent hybridization conditions.

SUMM . . . molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to ***human*** manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule of the present invention can be isolated from its natural source or can be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification,

cloning) or chemical synthesis. Isolated feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules can include, for example, natural allelic variants and nucleic acid molecules modified by nucleotide insertions, deletions, substitutions,. . . a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode a feline ***Fc*** .epsilon.R.alpha. protein of the present invention or to form stable hybrids under stringent conditions with natural gene isolates.

SUMM A feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule homolog can be produced using a number of methods known to those skilled in the art (see,. . . mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with a feline ***Fc*** .epsilon.R.alpha. gene or by screening for function of a protein encoded by the nucleic acid molecule (e.g., ability of a feline ***Fc*** .epsilon.R.alpha. protein to bind IgE).

SUMM . . . isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one feline ***Fc*** .epsilon.R.alpha. protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers. . . interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a feline ***Fc*** .epsilon.R.alpha. protein.

SUMM One embodiment of the present invention is a feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule that hybridizes under stringent hybridization conditions with nucleic acid molecule nfelFc.sub..epsilon. R.alpha..sub.1069 and preferably with a nucleic. . .

SUMM . . . acid sequences reported in GenBank indicates that SEQ ID NO:1 showed the most homology, i.e., about 77% identity a canine ***Fc*** ***epsilon*** ***receptor*** alpha chain gene.

SUMM Preferred feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules include nucleic acid molecules having a nucleic acid sequence that is at least about 80%, preferably at. . .

SUMM . . . ID NO:11, SEQ ID NO:14, SEQ ID NO:15 and/or SEQ ID NO:16, that is capable of hybridizing to a feline ***Fc*** .epsilon.R.alpha. gene of the present invention, as well as allelic variants thereof. A more preferred nucleic acid molecule includes the nucleic. . .

SUMM Knowing the nucleic acid sequences of certain feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of. . . (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules from other cats. Such nucleic acid molecules can be obtained in a variety of ways including screening. . .

SUMM . . . conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising feline ***Fc*** .epsilon.R.alpha. genes or other feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of. . . for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules or therapeutic reagents to inhibit feline ***Fc*** .epsilon.R.alpha. protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also includes the. . .

SUMM . . . is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulation of feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules of the present invention.

SUMM . . . the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed feline ***Fc*** .epsilon.R.alpha. protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences. . .

SUMM . . . that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include nfelFc.sub..epsilon. R.alpha..sub.1069, nfelFc.sub..epsilon.. . .

SUMM . . . production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of

producing feline ***Fc*** .epsilon.R.alpha. proteins of the present invention or can be capable of producing such proteins after being transformed with at least one. . . cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., ***human*** , murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK.sup.31 cells and/or. . .

SUMM Isolated feline ***Fc*** .epsilon.R.alpha. proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins,. . . permit protein production. An effective medium refers to any medium in which a cell is cultured to produce a feline ***Fc*** .epsilon.R.alpha. protein of the present invention. Such a medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources,. . .

SUMM The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to a feline ***Fc*** .epsilon.R.alpha. protein of the present invention or a mimetope thereof (i.e., anti-feline ***Fc*** .epsilon.R.alpha. antibodies). As used herein, the term "selectively binds to" a feline ***Fc*** .epsilon.R.alpha. protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof. . . in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., ibid. An anti-feline ***Fc*** .epsilon.R.alpha. antibody preferably selectively binds to a feline ***Fc*** .epsilon.R.alpha. protein in such a way as to reduce the activity of that protein.

SUMM . . . antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce feline ***Fc*** .epsilon.R.alpha. proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not. . .

SUMM . . . are within the scope of the present invention. For example, such antibodies can be used (a) as tools to detect ***Fc*** ***epsilon*** ***receptor*** in the presence or absence of IgE and/or (b) as tools to screen expression libraries and/or to recover desired proteins. . . proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target cytotoxic agents to cells having ***Fc*** ***epsilon*** ***receptors*** such as those disclosed herein in order to directly kill such cells. Targeting can be accomplished by conjugating (i.e., stably. . . in the art. Suitable cytotoxic agents are known to those skilled in the art. Antibodies of the present invention, including ***Fc*** .epsilon.R.alpha.-binding portions thereof, can also be used, for example, to inhibit binding of IgE to ***Fc*** ***epsilon*** ***receptors*** , to produce anti-feline ***Fc*** .epsilon.R.alpha. idiotypic antibodies, to purify cells having feline ***Fc*** .epsilon.R.alpha. proteins, to stimulate intracellular signal transduction through a feline ***Fc*** .epsilon.R.alpha. and to identify cells having feline ***Fc*** .epsilon.R.alpha. proteins.

SUMM A feline ***Fc*** .epsilon.R.alpha. molecule of the present invention can include chimeric molecules comprising a portion of a feline ***Fc*** .epsilon.R.alpha. molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the ***Fc*** .epsilon.R.alpha. molecule portion binds to IgE in essentially the same manner as a ***Fc*** .epsilon.R.alpha. molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of an. . .

SUMM A feline ***Fc*** .epsilon.R.alpha. molecule of the present invention can be contained in a formulation, herein referred to as a ***Fc*** .epsilon.R.alpha. molecule formulation. For example, a feline ***Fc*** .epsilon.R.alpha. molecule can be combined with a buffer in which the feline ***Fc*** .epsilon.R.alpha. molecule is solubilized, and/or with a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a feline ***Fc*** .epsilon.R.alpha. molecule can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate. . . not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be mixed with feline ***Fc*** .epsilon.R.alpha. molecules or conjugated (i.e., attached) to feline ***Fc***

.epsilon.R.alpha. molecules in such a manner as to not substantially interfere with the ability of the feline ***Fc*** .epsilon.R.alpha. molecules to selectively bind to IgE.

SUMM A feline ***Fc*** .epsilon.R.alpha. protein of the present invention can be bound to the surface of a cell comprising the feline ***Fc*** .epsilon.R.alpha. protein. A preferred feline ***Fc*** .epsilon.R.alpha. protein-bearing cell includes a recombinant cell comprising a nucleic acid molecule encoding a feline ***Fc*** .epsilon.R.alpha. protein of the present invention. A more preferred recombinant cell of the present invention comprises a nucleic acid molecule that. . .

SUMM In addition, a feline ***Fc*** .epsilon.R.alpha. molecule formulation of the present invention can include not only a feline ***Fc*** .epsilon.R.alpha. molecule but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers. . .

SUMM . . . of the present invention is a method to detect IgE which includes the steps of: (a) contacting an isolated feline ***Fc*** .epsilon.R.alpha. molecule with a putative IgE-containing composition under conditions suitable for formation of a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex; and (b) detecting the presence of IgE by detecting the feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex. Presence of such a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex indicates that the animal is producing IgE. Preferred IgE to detect using a feline ***Fc*** .epsilon.R.alpha. molecule include feline IgE, canine IgE, equine IgE and ***human*** IgE, with feline IgE being particularly preferred. The present method can further include the step of determining whether an IgE complexed with a feline ***Fc*** .epsilon.R.alpha. protein is heat labile. Preferably, a heat labile IgE is determined by incubating an IgE at about 56.degree. C. for. . . or heartworm allergens. Moreover, the inventors believe that identification of heat labile IgE and non-heat labile IgE using a feline ***Fc*** .epsilon.R.alpha. protein suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE antibodies. As such, a feline ***Fc*** .epsilon.R.alpha. protein of the present invention may be useful for detecting molecules bound by the feline ***Fc*** .epsilon.R.alpha. protein but not identical to a known IgE.

SUMM . . . used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with a feline ***Fc*** .epsilon.R.alpha. molecule. Formation of a complex between a feline ***Fc*** .epsilon.R.alpha. molecule and an IgE refers to the ability of the feline ***Fc*** .epsilon.R.alpha. molecule to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between a feline ***Fc*** .epsilon.R.alpha. molecule and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures,. . .

SUMM . . . are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between feline ***Fc*** .epsilon.R.alpha. molecule and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in. . .

SUMM . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the feline ***Fc*** .epsilon.R.alpha. molecule or to a reagent that selectively binds to the feline ***Fc*** .epsilon.R.alpha. molecule or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable. . . compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin available from Pierce, Rockford, Ill.). According to the present invention, a ***detectable*** ***marker*** can be connected to a feline ***Fc*** .epsilon.R.alpha. molecule using, for example, chemical conjugation or recombinant DNA technology (e.g.,

connection of a fusion segment such as that described. . . . binding domain; an immunoglobulin binding; a sugar binding domain; and a "tag" domain). Preferably a carbohydrate group of the feline ***Fc*** .epsilon.R.alpha. molecule is chemically conjugated to biotin.

SUMM In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a feline ***Fc*** .epsilon.R.alpha. molecule that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a feline ***Fc*** .epsilon.R.alpha. molecule includes, but is not limited to, a radioactive label, a fluorescent label, an enzyme label, a chemiluminescent label, a chromophoric label or a ligand. A ***detectable*** ***marker*** is conjugated to a feline ***Fc*** .epsilon.R.alpha. molecule in such a manner as not to block the ability of the feline ***Fc*** .epsilon.R.alpha. molecule to bind to the IgE being detected. Preferably, a carbohydrate group of a feline ***Fc*** .epsilon.R.alpha. molecule is conjugated to biotin.

SUMM In another embodiment, a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex is detected by contacting a putative IgE-containing composition with a feline ***Fc*** .epsilon.R.alpha. molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the feline ***Fc*** .epsilon.R.alpha. molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, an antigen, an antibody and a lectin, depending upon which portion of the feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex is being detected. Preferred indicator molecules that are antibodies include, for example, anti-IgE antibodies and anti-feline ***Fc*** .epsilon.R.alpha. antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a feline ***Fc*** .epsilon.R.alpha. protein of the present invention produced in insect cells. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

SUMM In one preferred embodiment, a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex is detected by contacting the complex with an indicator molecule that selectively binds to a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention. Examples of such indicator molecule includes, but are not limited to, an antibody that selectively binds to a feline ***Fc*** .epsilon.R.alpha. molecule (referred to herein as an anti-feline ***Fc*** .epsilon.R.alpha. antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to a feline ***Fc*** .epsilon.R.alpha. molecule. A feline ***Fc*** .epsilon.R.alpha. molecule conjugated to biotin is preferably detected using streptavidin, more preferably using ImmunoPure.RTM. NeutrAvidin (available from Pierce, Rockford, Ill.).

SUMM In another preferred embodiment, a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex is detected by contacting the complex with indicator molecule that selectively binds to an IgE antibody (referred to. . . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., a ***Fc*** receptor), and an antibody-binding complement protein. A preferred indicator molecule includes an anti-feline IgE antibody. As used herein, an anti-IgE. . . .

SUMM . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

SUMM A preferred immunoabsorbent assay method includes a step of either: (a) immobilizing a feline ***Fc*** .epsilon.R.alpha. molecule on a substrate prior to contacting a feline ***Fc*** .epsilon.R.alpha. molecule with a putative IgE-containing composition to form a feline ***Fc*** .epsilon.R.alpha. molecule-immobilized substrate; and (b) binding a putative IgE-containing composition on a substrate prior to contacting a feline ***Fc*** .epsilon.R.alpha. molecule with a putative IgE-containing composition to form a putative IgE-containing composition-bound substrate. Preferably, the substrate includes a non-coated substrate, a feline ***Fc*** .epsilon.R.alpha. molecule-immobilized substrate, an antigen-immobilized substrate or an anti-IgE antibody-immobilized substrate.

SUMM . . . whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, a feline ***Fc***

.epsilon.R.alpha. molecule of the present invention is used as a capture molecule when the feline ***Fc*** .epsilon.R.alpha. molecule is bound on a substrate. Alternatively, a feline ***Fc*** .epsilon.R.alpha. molecule is used as an indicator molecule when the feline ***Fc*** .epsilon.R.alpha. molecule is not bound on a substrate. Suitable molecules for use as capture molecules or indicator molecules include, but are not limited to, a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

SUMM . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

SUMM . . . the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is a feline ***Fc*** .epsilon.R.alpha. molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

SUMM In one embodiment, a feline ***Fc*** .epsilon.R.alpha. molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or. . . biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the feline ***Fc*** .epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a calorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . .

SUMM . . . if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. A feline ***Fc*** .epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the feline ***Fc*** .epsilon.R.alpha. molecule and the anti-IgE antibody:IgE complex. Preferably, the feline ***Fc*** .epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess feline ***Fc*** .epsilon.R.alpha. molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

SUMM . . . non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A feline ***Fc*** .epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the feline ***Fc*** .epsilon.R.alpha. molecule and the IgE. Preferably, the feline ***Fc*** .epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess feline ***Fc*** .epsilon.R.alpha. molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

SUMM . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . . which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case a feline ***Fc*** .epsilon.R.alpha. molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture reagent is. . .

SUMM . . . used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a feline ***Fc*** .epsilon.R.alpha. molecule as described above, the labeling reagent

impregnated within the support structure in a labeling zone; and (c) a capture. . . .

SUMM . . . in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention and an isolated IgE known to bind to the feline ***Fc*** .epsilon.R.alpha. molecule. The absence of binding of the feline ***Fc*** .epsilon.R.alpha. molecule to the known IgE indicates the presence of IgE in the putative IgE-containing composition. The known IgE is preferably conjugated to a ***detectable*** ***marker*** .

SUMM . . . IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising a feline ***Fc*** .epsilon.R.alpha. protein and a means for detecting an IgE. Suitable and preferred feline ***Fc*** .epsilon.R.alpha. protein are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the feline ***Fc*** .epsilon.R.alpha. protein or to an IgE. A preferred kit of the present invention further comprises a detection means including one or. . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a feline ***Fc*** .epsilon.R.alpha. protein (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including canines, felines and/or equines.

SUMM . . . invention is a general allergen kit comprising an allergen common to all regions of the United States and a feline ***Fc*** .epsilon.R.alpha. protein of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that are. . .

SUMM . . . as cod, halibut or and tuna, egg, milk, Brewer's yeast, whole wheat, corn, soybean, cheese and rice, and a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

SUMM . . . present invention is a therapeutic composition that, when administered to an animal in an effective manner, is capable of reducing ***Fc*** receptor mediated reactions associated with diseases related to biological responses involving ***Fc*** receptor function. A therapeutic composition of the present invention can include: an isolated feline ***Fc*** .epsilon.R.alpha. protein, or homolog thereof; a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a feline ***Fc*** .epsilon.R.alpha. gene; an isolated antibody that selectively binds to a feline ***Fc*** .epsilon.R.alpha. protein; and/or an inhibitor that interferes with formation of a complex between a feline ***Fc*** .epsilon.R.alpha. protein and IgE.

SUMM One embodiment of a therapeutic composition of the present invention is a therapeutic compound comprising a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention, that binds to an IgE. According to the present invention, a feline ***Fc*** .epsilon.R.alpha. molecule competes for IgE with naturally-occurring ***Fc*** ***epsilon*** ***receptors*** , particularly those on mastocytoma cells, mast cells or basophils, so that IgE is bound to the administered feline ***Fc*** .epsilon.R.alpha. molecule and thus is unable to bind to ***Fc*** ***epsilon*** ***receptor*** on a cell, thereby inhibiting mediation of a biological response. Preferred feline ***Fc*** .epsilon.R.alpha. molecule for use in a therapeutic composition comprises a feline ***Fc*** .epsilon.R.alpha. protein, or homolog thereof, as described herein, particularly a fragment thereof, which binds to IgE. Feline ***Fc*** .epsilon.R.alpha. molecules for use in a therapeutic composition can be in a monovalent and/or multivalent form, so long as the feline ***Fc*** .epsilon.R.alpha. molecule is capable of binding to IgE. A more preferred feline ***Fc*** .epsilon.R.alpha. molecule for use in a therapeutic composition includes a soluble fragment of a feline ***Fc*** .epsilon.R.alpha. protein. A preferred feline ***Fc*** .epsilon.R.alpha. protein is encoded by nfelFc.sub..epsilon. R.alpha..sub.522 and an even more preferred feline ***Fc*** .epsilon.R.alpha. protein is PfelFc.sub..epsilon. R.alpha..sub.174.

SUMM . . . therapeutic composition of the present invention comprises a therapeutic compound that interferes with the formation of a complex

between feline ***Fc*** .epsilon.R.alpha. protein and IgE, usually by binding to or otherwise interacting with or otherwise modifying the feline ***Fc*** .epsilon.R.alpha. protein's IgE binding site. Feline ***Fc*** .epsilon.R.alpha. protein inhibitors can also interact with other regions of the feline ***Fc*** .epsilon.R.alpha. protein to inhibit feline ***Fc*** .epsilon.R.alpha. protein activity, for example, by allosteric interaction. An inhibitor of a feline ***Fc*** .epsilon.R.alpha. protein can interfere with ***Fc*** .epsilon.R.alpha. protein and IgE complex formation by, for example, preventing formation of a ***Fc*** .epsilon.R.alpha. protein and IgE complex or disrupting an existing ***Fc*** .epsilon.R.alpha. protein and IgE complex causing the ***Fc*** .epsilon.R.alpha. protein and IgE to dissociate. An inhibitor of a feline ***Fc*** .epsilon.R.alpha. protein is usually a relatively small. Preferably, a feline ***Fc*** .epsilon.R.alpha. protein inhibitor of the present invention is identified by its ability to bind to, or otherwise interact with, a feline ***Fc*** .epsilon.R.alpha. protein, thereby interfering with the formation of a complex between a feline ***Fc*** .epsilon.R.alpha. protein and IgE.

SUMM Preferred inhibitors of a feline ***Fc*** .epsilon.R.alpha. protein of the present invention include, but are not limited to, a substrate analog of a feline ***Fc*** .epsilon.R.alpha. protein, a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein, a soluble (i.e., secreted form of a feline ***Fc*** .epsilon.R.alpha. protein) portion of a feline ***Fc*** .epsilon.R.alpha. protein that binds to IgE, and other molecules that bind to a feline ***Fc*** .epsilon.R.alpha. protein (e.g., to an allosteric site) in such a manner that IgE-binding activity of the feline ***Fc*** .epsilon.R.alpha. protein is inhibited. A feline ***Fc*** .epsilon.R.alpha. protein substrate analog refers to a compound that interacts with (e.g., binds to, associates with, modifies) the IgE-binding site of a feline ***Fc*** .epsilon.R.alpha. protein. A preferred feline ***Fc*** .epsilon.R.alpha. protein substrate analog inhibits IgE-binding activity of a feline ***Fc*** .epsilon.R.alpha. protein. Feline ***Fc*** .epsilon.R.alpha. protein substrate analogs can be of any inorganic or organic composition, and, as such, can be, but are not limited to, peptides, nucleic acids, and peptidomimetic compounds. Feline ***Fc*** .epsilon.R.alpha. protein substrate analogs can be, but need not be, structurally similar to a feline ***Fc*** .epsilon.R.alpha. protein's natural substrate (e.g., IgE) as long as they can interact with the active site (e.g., IgE-binding site of that feline ***Fc*** .epsilon.R.alpha.). Feline ***Fc*** .epsilon.R.alpha. protein substrate analogs can be designed using computer-generated structures of feline ***Fc*** .epsilon.R.alpha. proteins of the present invention or computer structures of, for example, the ***Fc*** domain of IgE. Substrate analogs can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides, peptidomimetic. . . inorganic or organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., a feline ***Fc*** .epsilon.R.alpha. protein or anti-feline ***Fc*** .epsilon.R.alpha. idiotypic antibody). A preferred feline ***Fc*** .epsilon.R.alpha. protein substrate analog is a peptidomimetic compound (i.e., a compound that is structurally and/or functionally similar to a natural substrate of a feline ***Fc*** .epsilon.R.alpha. protein of the present invention, particularly to the region of the substrate that binds to a feline ***Fc*** .epsilon.R.alpha. protein, but that inhibits IgE binding upon interacting with the IgE binding site).

SUMM Feline ***Fc*** .epsilon.R.alpha. molecules, as well as other inhibitors and therapeutic compounds, can be used directly as compounds in compositions of the present. . .

SUMM In one embodiment, a therapeutic composition of the present invention can be used to reduce a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response in an animal by administering such a composition to an animal. Preferably, an animal is treated by administering. . . therapeutic composition of the present invention in such a manner that a therapeutic compound (e.g., an inhibitor of a feline ***Fc*** .epsilon.R.alpha. protein, an anti-feline ***Fc*** .epsilon.R.alpha. antibody, an inhibitor of IgE, or nucleic acid molecules encoding feline ***Fc*** .epsilon.R.alpha. proteins) binds to an IgE or a ***Fc*** ***epsilon*** ***receptor*** in the

animal. Such administration could be by a variety of routes known to those skilled in the art including,. . .

SUMM Compositions of the present invention can be administered to any animal having a ***Fc*** ***epsilon*** ***receptor*** or an IgE that binds to a therapeutic compound of the present invention or to a protein expressed by a. . .

SUMM . . . suitable liquid as a suspension or solution for injection. Thus, in a non- liquid formulation, the excipient can comprise dextrose, ***human*** serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

SUMM . . . the blood of an animal at a constant rate sufficient to attain therapeutic dose levels of the composition to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in the animal. As used herein, a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response refers to cellular responses that occur when ***Fc*** ***epsilon*** ***receptor*** is complexed with IgE. For example, a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response includes release of biological mediators, such as histamine, prostaglandins and/or proteases, that can trigger clinical symptoms of allergy.. . .

SUMM . . . skill in the art in accordance with the given condition of a patient. For example, to regulate an antigen- specific ***Fc*** ***epsilon*** ***receptor*** -mediated response, a therapeutic composition may be administered more frequently when an antigen is present in a patient's environment in high. . .

SUMM . . . can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a feline ***Fc*** .epsilon.R.alpha. protein or a feline ***Fc*** .epsilon.R.alpha. RNA (e.g., antisense RNA, ribozyme, triple helix forms or RNA drug) in the animal. Nucleic acid molecules can be delivered. . .

SUMM . . . the recipient animal and directs the production of a protein or RNA nucleic acid molecule that is capable of reducing ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in the animal. For example, a recombinant virus comprising a feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule of the present invention is administered according to a protocol that results in the animal producing an amount of protein or RNA sufficient to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. A preferred single dose of a recombinant virus of the present invention is from about 1.times.10.sup.4 to about. . .

SUMM . . . a therapeutic composition of the present invention includes recombinant cells of the present invention that comprises at least one feline ***Fc*** .epsilon.R.alpha. of the present invention. Preferred recombinant cells for this embodiment include Salmonella, E. coli, Listeria, Mycobacterium, S. frugiperda, yeast, (including. . .

SUMM . . . an animal an effective amount of a therapeutic composition selected from the group consisting of an inhibitor of a feline ***Fc*** .epsilon.R.alpha. and a feline ***Fc*** .epsilon.R.alpha. protein (including homologs), wherein said feline ***Fc*** .epsilon.R.alpha. is capable of binding to IgE. Suitable therapeutic compositions and methods of administration methods are disclosed herein. According to the. . . invention, a therapeutic composition and method of the present invention can be used to prevent or alleviate symptoms associated with ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

SUMM The efficacy of a therapeutic composition of the present invention to effect ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses can be tested using standard methods for detecting ***Fc*** receptor-mediated immunity including, but not limited to, immediate hypersensitivity, delayed hypersensitivity, antibody-dependent cellular cytotoxicity (ADCC), immune complex activity, mitogenic activity,. . .

SUMM An inhibitor of feline ***Fc*** .epsilon.R.alpha. activity can be identified using feline ***Fc*** .epsilon.R.alpha. proteins of the present invention by determining the ability of an inhibitor to prevent or disrupt complex formation between a feline ***Fc*** .epsilon.R.alpha. protein and IgE. One embodiment of the present invention is a method to identify a compound capable of inhibiting feline ***Fc*** .epsilon.R.alpha. activity. Such a method includes the steps of (a) contacting (e.g., combining, mixing) an isolated feline ***Fc*** .epsilon.R.alpha. protein with a putative inhibitory compound

under conditions in which, in the absence of the compound, the feline ***Fc*** .epsilon.R.alpha. protein has IgE binding activity, and (b) determining if the putative inhibitory compound inhibits the IgE binding activity. Putative inhibitory. . .

SUMM The present invention also includes a test kit to identify a compound capable of inhibiting feline ***Fc*** .epsilon.R.alpha. activity. Such a test kit includes: an isolated feline ***Fc*** .epsilon.R.alpha. protein having IgE binding activity or a complex of feline ***Fc*** .epsilon.R.alpha. protein and IgE; and a means for determining the extent of inhibition of IgE binding activity in the presence of. . .

DETD This example describes the isolation, by DNA hybridization, of a nucleic acid molecule encoding a feline ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .epsilon.R.alpha.) protein from Felis domesticus.

DETD A feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule was isolated from a feline (Felis domesticus) mastocytoma cDNA library by hybridizing the library with a mixture of .sup.32 P-labeled cDNA molecules encoding ***human*** and canine ***Fc*** ***epsilon*** ***receptor*** alpha chains, respectively. A feline mastocytoma cDNA library was prepared as follows. Total RNA was extracted from approximately 1.5 grams. . . the feline mastocytoma cDNA library was screened, using duplicate plaque lifts, with a mixture of .sup.32 P-labeled cDNAs encoding the ***human*** ***Fc*** ***epsilon*** ***receptor*** alpha chain (Kochan et al., Nucleic Acids Res., 16:3584, 1988) and the canine ***Fc*** ***epsilon*** ***receptor*** alpha chain (Hayashi et al., GenBank accession number D 16413, 1993), repsectively. A plaque purified clone identified using the above. . .

DETD This Example demonstrates the production of secreted feline ***Fc*** .epsilon.R.alpha. chain protein in eukaryotic cells.

DETD To produce a secreted form of the extracellular domain of the feline ***Fc*** .epsilon.R.alpha. chain, the hydrophobic transmembrane domain and the cytoplasmic tail of the feline ***Fc*** .epsilon.R.alpha. chain encoded by nfelFc.sub..epsilon. R.alpha..sub.1069 were removed as follows. A feline ***Fc*** .epsilon.R.alpha. chain extracellular domain nucleic acid molecule-containing a fragment of about 597 nucleotides was PCR amplified from nfelFc.sub..epsilon. R.alpha..sub.1069 using a. . . pVL-nfelFc.sub..epsilon. R.alpha..sub.597. Nucleic acid molecule Bv-nfelFc.sub..epsilon. R.alpha..sub.597 contained an about 597 nucleotide fragment encoding the extracellular domain of the feline ***Fc*** .epsilon.R.alpha. chain, extending from about nucleotide 65 through about 661 of SEQ ID NO:1, denoted herein as nucleic acid molecule nfelFc.sub..epsilon.. . . acid sequence denoted SEQ ID NO:11. Translation of SEQ ID NO:11 indicates that nucleic acid molecule nfelFc.sub..epsilon. R.alpha..sub.597, encodes a ***Fc*** .epsilon.R.alpha. protein of about 199 amino acids, referred to herein as PfelFc.sub..epsilon. R.alpha..sub.199, having amino acid sequence SEQ ID NO:12. Nucleic acid molecule nfelFc.sub..epsilon. R.alpha..sub.597 encodes a secretable form of the feline ***Fc*** .epsilon.R.alpha. chain. The processed protein product encoded by nfelFc.sub..epsilon. R.alpha..sub.597 is about 174 amino acids and does not possess a leader. . .

DETD . . . frugiperda:pVL-nfelFc.sub..epsilon. R.alpha..sub.597. S. frugiperda:pVL-nfelFc.sub..epsilon. R.alpha..sub.597 is cultured using techniques known to those skilled in the art to produce a feline ***Fc*** .epsilon.R.alpha. protein PfelFc.sub..epsilon. R.alpha..sub.199.

CLM What is claimed is:
7. A method to produce a ***Fc*** .epsilon.R.alpha. protein, said method comprising culturing a cell transformed with a nucleic acid molecule that encodes a protein comprising an amino. . .

L13 ANSWER 20 OF 24 USPATFULL on STN
AN 2000:88312 USPATFULL
TI Asthma related genes
IN Brooks-Wilson, Angela R., San Diego, CA, United States
Buckler, Alan, Cardiff, CA, United States
Cardon, Lon, San Diego, CA, United States
Carey, Alisoun H., San Diego, CA, United States
Galvin, Margaret, Encinitas, CA, United States

Miller, Andrew, San Diego, CA, United States
North, Michael, San Diego, CA, United States

PA AxyS Pharmaceuticals, Inc., South San Francisco, CA, United States (U.S. corporation)

PI US 6087485 20000711

AI US 1998-9913 19980121 (9)

PRAI US 1997-35663P 19970121 (60)
US 1997-51432P 19970701 (60)

DT Utility

FS Granted

EXNAM Primary Examiner: Yucel, Remy

LREP Sherwood, Pamela J., Borden, Paul A.Bozicevic, Field & Francis LLP

CLMN Number of Claims: 7

ECL Exemplary Claim: 1

DRWN 1 Drawing Figure(s); 1 Drawing Page(s)

LN.CNT 6394

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A genetic locus associated with asthma is identified. The genes within the locus, ASTH1I and ASTH1J, and the regulatory sequences of the locus are characterized. The genes are used to produce the encoded proteins; in screening for compositions that modulate the expression or function of ASTH1 proteins; and in studying associated physiological pathways. The DNA is further used as a diagnostic for genetic predisposition to asthma.

SUMM In recent years thousands of ***human*** genes have been cloned. In many cases, gene discovery has been based on prior knowledge about the corresponding protein, such. . . genes in these cases are identified based on knowledge of molecular level protein properties. For a large number of important ***human*** genes, however, there is little or no biochemical data concerning the encoded product. For example, genes that predispose to ***human*** diseases, such as cystic fibrosis, Huntington's disease, etc. are of interest because of their phenotypic effect. Biochemical characterization of such. . .

SUMM The association of a polymorphism for the ***Fc*** .epsilon.RI-.beta. gene and risk of atopy is described in Hill et al. (1995) B.M.J. 311:776; Hill and Cookson (1996) ***Human*** Mol. Genet. 5:959; and Shirakawa et al. (1994) Nature Genetics 7:125; an association of ***Fc*** .epsilon.RI-.beta. with bronchial hyperreactivity is described in van Herwerden (1995) The Lancet 346:1262.

SUMM Collections of polymorphic markers from throughout the ***human*** genome have been tested for linkage to asthma, described in Meyers et al. (1996) Am. J. Hum. Genet. 59:A228 and Daniels et al. (1996) Nature 383:247-250. No linkage to ***human*** chromosome 11p was detected in these studies.

SUMM ***Human*** genes associated with a genetic predisposition to asthma are provided. The genes, herein termed ASTH1I and ASTH1J, are located close to each other on ***human*** chromosome 11p, have similar patterns of expression, and common sequence motifs. The nucleic acid compositions are used to produce the. . .

DETD The ***human*** ASTH1I and ASTH1J gene sequences are provided, as are the genomic sequences 5' to ASTH1J. The major sequences of interest. . .

DETD The ASTH1 locus has been mapped to ***human*** chromosome 11p. The traits for a positive response to methacholine challenge and a clinical history of asthma were shown to. . .

DETD . . . is provided in SEQ ID NO:326, and the amino acid sequence is provided in SEQ ID NO:327. The mouse and ***human*** proteins have 88.4% identity throughout their length. The match in the ets domain is 100%. The mouse cDNA was identified by hybridization of a full-length ***human*** cDNA to a mouse lung cDNA library (Stratagene).

DETD . . . one can isolate homologous or related genes. The source of homologous genes may be any species, e.g. primate species, particularly ***human*** ; rodents, such as rats and mice, canines, felines, bovines, ovines, equines, yeast, Drosophila, Caenhorabditis, etc.

DETD . . . be modulated by the patient genotype in other genes related to asthma and atopy, including, but not limited to, the ***Fc*** . ***epsilon*** . ***receptor*** , Class I and Class II HLA antigens, T cell receptor and immunoglobulin genes, cytokines and cytokine receptors, and the like.

DETD . . . length. Repeats can be simple or complex. The flanking sequences U and U' uniquely identify the microsatellite locus within the

human genome. U and U' are at least about 18 nucleotides in length, and may extend several hundred bases up to. . .

DETD . . . the gene include plasmids and viral vectors. Of particular interest are retroviral-based vectors, e.g. Moloney murine leukemia virus and modified ***human*** immunodeficiency virus; adenovirus vectors, etc. that are maintained transiently or stably in mammalian cells. A wide variety of vectors can. . .

DETD . . . into a chromosome. Many strong promoters are known in the art, including the .beta.-actin promoter, SV40 early and late promoters, ***human*** cytomegalovirus promoter, retroviral LTRs, methallothionein responsive element (MRE), tetracycline-inducible promoter constructs, etc.

DETD The subject nucleic acids can be used to generate genetically modified non- ***human*** animals or site specific gene modifications in cell lines. The term "transgenic" is intended to encompass genetically modified animals having. . .

DETD . . . anti-sense ASTH1, which will block ASTH1 expression, expression of dominant negative ASTH1 mutations, and over-expression of a ASTH1 gene. A ***detectable*** ***marker*** , such as lac Z may be introduced into the ASTH1 locus, where upregulation of ASTH1 expression will result in an. . .

DETD . . . complement defined genetic lesions in order to determine the physiological and biochemical pathways involved in ASTH1 function. A number of ***human*** genes have been shown to complement mutations in lower eukaryotes.

DETD . . . ASTH1 function may stimulate bronchial reactivity. Of particular interest are screening assays for agents that have a low toxicity for ***human*** cells. A wide variety of assays may be used for this purpose, including labeled in vitro protein-protein binding assays, protein-DNA. . .

DETD . . . developed at Oxford (Reed et al. (1994) Nature Genetics 7:390), Genethon (Dib et al. (1996) Nature 380:152) and the Cooperative ***Human*** Linkage Center (CHLC, Murray et al. (1994) Science 265:2049). Markers with heterozygosity values of 0.75 or greater were selected to cover all the ***human*** chromosomes, as well as for ease of genotyping and size of PCR product for multiplexing of markers on gels. Fifteen. . .

DETD . . . of water was added and unincorporated nucleotide was removed by Quick Spin Column (Boehringer Mannheim). 23 .mu.l of 11.2 mg/ml ***human*** placental DNA (Sigma) and 36 .mu.l of 0.5 M Na.sub.2 HPO.sub.4, pH 6.0 were added to the approximately 150 .mu.l. . . (1992) Proc. Natl. Acad. Sci. 89:8794; purchased from Research Genetics) or chromosome 11 cosmid [Resource Center/Primary Database of the German ***Human*** Genome Project, Berlin; Lehrach et al. (1990), In Davies, K. E. and Tilghman, S. M. (eds.), Genome Analysis Volume 1:. . .

DETD . . . on DAPI banded chromosomes. In Methods of Molecular Biology: In Situ Hybridisation Protocols (K. H. A. Choo, ed.) pp. 35-49. ***Human*** Press, Clifton, N.J.). High resolution FISH, or DIRVISH, was used to map the relative positions of two or more clones. . . (1993) Nature Genet. 5:17. Briefly, slides containing stretched DNA were prepared by adding 2 .mu.l of a suspension of normal ***human*** lymphoblast cells at one end of a glass slide and allowing to dry. 8 .mu.l lysis buffer (0.5% SDS, 50. . .

DETD . . . were filled by screening a gridded BAC library with the end clone probes or by screening DNA pools of a ***human*** genomic PAC library (loannou et al. (1994) Nature Genetics 6:84; licensed from Health Research, Inc.) by PCR using primers designed. . .

DETD A genome scan was performed using polymorphic microsatellite markers from throughout the ***human*** genome, and DNA isolated from blood samples drawn from the inhabitants of Tristan da Cunha. Linkage analysis, an established statistical. . . and markers relative to other markers, was applied to verify the marker orders and relative distances between markers on all ***human*** chromosomes, in the Tristan da Cunha population. Linkage analysis can detect cosegregation of a marker with disease, and was used. . .

DETD . . . size purified artificial chromosomes were used as hybridization probes to identify BAC and cosmid clones. Gridded filters of a 3.times. ***human*** genomic BAC library and of a ***human*** chromosome 11-specific cosmid library were hybridized with radiolabeled purified YAC. Clones corresponding to the grid coordinates of the positives were. . .

DETD . . . map produced had three gaps which were subsequently filled by end cloning and hybridization of the end clones to a ***human*** genomic PAC library. Genetic refinement of the ASTH1 region had occurred concurrently with mapping, rendering it unnecessary to extend the. . .

DETD . . . the Utah genome center, see: The Utah Marker Development Group (1995) Am. J. Hum. Genet. 57:619; c = the cooperative ***Human*** Lineage Center.

DETD ***Human*** multi-tissue Northern blots were probed with PCR products of masterplate clones. In some cases, exon trapping clones did not detect. . .

DETD . . . NET, SAP-1, NERF and FLI. Secondary structure analysis and comparison of the protein sequence to the crystal structure of the ***human*** ETS1-DNA complex (Werner et al. (1995) Cell 83:761) confirmed that it has a winged helix turn helix motif characteristic of. . .

DETD . . . repeat elements such as Alu or LINEs. Inserts from these clones were used as probes on Southern blots of EcoRI-digested ***human*** , mouse and pig or cow genomic DNA. Probes that produced discrete bands in more than one species were considered conserved.

DETD . . . gene transcriptional initiation. None of these sites are found when the primer extension analysis is performed using mRNA isolated from ***human*** lung fibroblasts that do not express ASTH1J.

DETD . . . screening a mouse 129Sv genomic phage library (Stratagene) with the 443 bp BamHI-SmaI fragment from the 5' region of the ***human*** asth1-J cDNA clone PA1001A as probe. The 23 kb insert in MW1-J was sequenced.

DETD The data presented above demonstrate that ASTH1I and ASTH1J are novel ***human*** genes linked to a history of clinical asthma and bronchial hyperreactivity in two asthma cohorts, the population of Tristan da. . .

L13 ANSWER 21 OF 24 USPATFULL on STN

AN 2000:57620 USPATFULL

TI Method to detect canine IgE and kit therefor

IN Frank, Glenn R., Wellington, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States

PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)

PI US 6060326 20000509

AI US 1997-833488 19970407 (8)

DT Utility

FS Granted

EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Gabel, Gailene R.

LREP Heska Corporation

CLMN Number of Claims: 38

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 2232

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention includes a method to detect canine IgE using a canine ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon. R) to detect canine IgE antibodies in a biological sample from a canid. The present invention also relates to kits to perform such methods.

AB The present invention includes a method to detect canine IgE using a canine ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon. R) to detect canine IgE antibodies in a biological sample from a canid. The present invention also relates to kits. . .

SUMM Until the discovery of the present invention, detection of IgE in samples obtained from non- ***human*** animals has been hindered by the absence of suitable reagents for detection of IgE. Various compounds have been used to. . . other antibody idiotypes, such as gamma isotype antibodies. The discovery of the present invention includes the use of a canine ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon. R) molecule to detect the presence of IgE in a putative IgE-containing composition. Canine high affinity ***Fc*** .sub..epsilon. R consists of three protein chains, alpha, beta and gamma. Hayashi et al. have disclosed the nucleic acid sequence for the alpha chain (GenBank Accession No. D16413, submitted Jun. 8, 1993). A canine ***Fc*** .sub..epsilon. R molecule provides an advantage over, for example anti-IgE antibodies, to detect IgE because a canine

Fc .sub..epsilon. R molecule can bind to a canine IgE with more specificity (i.e., less idiotype cross-reactivity) and more sensitivity (i.e., affinity). . .

SUMM Thus, methods and kits are needed in the art that will provide specific detection of canine IgE using canine ***Fc*** .sub..epsilon. R.

SUMM One embodiment of the present invention is a method to detect canine IgE comprising: (a) contacting an isolated canine ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule with a putative canine IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon. R molecule:IgE complex; and (b) determining the presence of IgE by detecting the ***Fc*** .sub..epsilon. R molecule:IgE complex, the presence of the ***Fc*** .sub..epsilon. R molecule:IgE complex indicating the presence of IgE. In particular, the canine ***Fc*** .sub..epsilon. R molecule comprises at least a portion of a ***Fc*** .sub..epsilon. R alpha chain that binds to canine IgE.

SUMM . . . binding to the substrate; and (d) detecting the presence of the antigen:IgE complex by contacting antigen:IgE complex with a canine ***Fc*** .sub..epsilon. R molecule. In particular, the flea allergen is a flea saliva antigen.

SUMM . . . a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a canine ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and a means for detecting canine IgE. Another embodiment is a kit for detecting flea allergy dermatitis comprising a canine ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and a flea allergen.

SUMM The present invention relates to the discovery that purified high affinity canine ***Fc*** ***epsilon*** ***receptor*** (i.e., ***Fc*** .sub..epsilon. RI; referred to herein as ***Fc*** .sub..epsilon. R) can be used in canine epsilon immunoglobulin (referred to herein as IgE or IgE antibody)-based detection (e.g., diagnostic, screening) methods and kits. The use of canine ***Fc*** .sub..epsilon. R in diagnostic methods and kits is unexpected because the use of canine ***Fc*** .sub..epsilon. R avoids complications presented by use of antibodies that bind to IgE (i.e., anti-IgE antibodies). Such complications include, for example,. . .

SUMM One embodiment of the present invention is a method to detect a canine IgE using an isolated canine ***Fc*** .sub..epsilon. R molecule. It is to be noted that the term "a" entity or "an" entity refers to one or more. . .

SUMM According to the present invention, an isolated, or biologically pure, ***Fc*** .sub..epsilon. R molecule, is a molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the molecule has been purified. An isolated canine ***Fc*** .sub..epsilon. R molecule of the present invention can be obtained from its natural source (e.g., from a canine mast cell), can. . .

SUMM A ***Fc*** .sub..epsilon. R molecule (also referred to herein as ***Fc*** .sub..epsilon. R or ***Fc*** .sub..epsilon. R protein) of the present invention can be a full-length protein, a portion of a full-length protein or any homolog of such a protein, wherein the ***Fc*** .sub..epsilon. R molecule is capable of binding specifically to IgE. As used herein, a protein can be a polypeptide or a peptide. A ***Fc*** .sub..epsilon. R molecule of the present invention can comprise a complete ***Fc*** .sub..epsilon. R (i.e., alpha, beta and gamma ***Fc*** .sub..epsilon. R chains), an alpha ***Fc*** .sub..epsilon. R chain (also referred to herein as ***Fc*** .sub..epsilon. R .alpha. chain) or portions thereof. Preferably, a ***Fc*** .sub..epsilon. R molecule comprises at least a portion of a ***Fc*** .sub..epsilon. R .alpha. chain that binds to IgE, i.e., that is capable of forming an immunocomplex with an IgE constant region.

SUMM An isolated canine ***Fc*** .sub..epsilon. R molecule of the present invention, including a homolog, can be identified in a straight-forward manner by the ***Fc*** .sub..epsilon. R molecule's ability to form an immunocomplex with a canine IgE. Examples of ***Fc*** .sub..epsilon. R homologs include ***Fc*** .sub..epsilon. R proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide),. . .

SUMM ***Fc*** .sub..epsilon. R homologs can be the result of natural allelic variation or natural mutation. ***Fc*** .sub..epsilon. R

homologs of the present invention can also be produced using techniques known in the art including, but not limited. . .

SUMM According to the present invention, a preferred canine ***Fc*** .sub..epsilon. R .alpha. chain of the present invention is encoded by at least a portion of the nucleic acid sequence of the coding strand of a cDNA encoding a full-length ***Fc*** .sub..epsilon. R .alpha. chain protein represented herein as SEQ ID NO: 19, the portion at least encoding the IgE binding site of the ***Fc*** .sub..epsilon. R .alpha. chain protein. Other suitable canine ***Fc*** .sub..epsilon. R .alpha. chains useful in the present invention include those described herein in the Examples section. The double-stranded nucleic acid. . . by one skilled in the art and is shown herein as SEQ ID NO: 21) is referred to herein as ***Fc*** .sub..epsilon. R nucleic acid molecule ncFc.sub..epsilon. R.alpha.4.sub.991. Translation of SEQ ID NO: 19 suggests that nucleic acid molecule ncFc.sub..epsilon. R.alpha.4.sub.991, encodes a full-length ***Fc*** .sub..epsilon. R .alpha. chain protein of about 253 amino acids, referred to herein as PcFc.sub..epsilon. R.alpha.4.sub.253, represented by SEQ ID NO:. . . in the art to make modifications to the respective nucleic acid molecules and proteins to, for example, develop a canine ***Fc*** .sub..epsilon. R .alpha. chain protein with increased solubility and/or a truncated protein capable of detecting canine IgE, e.g., PcFc.sub..epsilon. R.alpha.4.sub.197, spanning. . .

SUMM Preferred ***Fc*** .sub..epsilon. R molecules include PcFc.sub..epsilon. R.alpha.4.sub.253, PcFc.sub..epsilon. R.alpha.4.sub.229, PcFc.sub..epsilon. R.alpha.4.sub.197, PcFc.sub..epsilon. R.alpha.4.sub.173 and allelic variants thereof, as well as PcFc.sub..epsilon. R.alpha..sub.197,. . . PcFc.sub..epsilon. R.alpha.3.sub.199 (which are disclosed in the Examples section) and allelic variants thereof. Preferred nucleic acid molecules to encode a ***Fc*** .sub..epsilon. R molecules include ncFc.sub..epsilon. R.alpha.4.sub.191, ncFc.sub..epsilon. R.alpha.4.sub.687, ncFc.sub..epsilon. R.alpha.4.sub.991, ncFc.sub..epsilon. R.alpha.4.sub.759 and allelic variants thereof, as well as ncFc.sub..epsilon. R.alpha.1.sub.609,. . . R.alpha.3.sub.597 (which are disclosed in the Examples section) and allelic variants thereof. A preferred nucleic acid sequence encoding a canine ***Fc*** .sub..epsilon. R molecule includes SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ. . .

SUMM An isolated canine ***Fc*** .sub..epsilon. R molecule protein of the present invention can be produced by culturing a cell capable of expressing the protein under. . . Suitable and preferred nucleic acid molecules with which to transform a cell are as disclosed herein for suitable and preferred ***Fc*** .sub..epsilon. R nucleic acid molecules per se. Particularly preferred nucleic acid molecules to include in recombinant cells of the present invention. . .

SUMM . . . nucleic acid molecule. Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing a canine ***Fc*** .sub..epsilon. R molecule protein of the present invention or can be capable of producing such proteins after being transformed with at. . .

SUMM . . . herein. Particularly preferred recombinant molecules include pVL-ncFc.sub..epsilon. R.alpha.4.sub.591, pVL-ncFc.sub..epsilon. R.alpha.1.sub.609, pVL-ncFc.sub..epsilon. R.alpha.2.sub.609, and pVL-ncFc.sub..epsilon. R.alpha.3.sub.617. Details regarding the production of ***Fc*** .sub..epsilon. R molecule nucleic acid molecule-containing recombinant molecules are disclosed herein. Particularly preferred recombinant cells of the present invention include S.. . .

SUMM A ***Fc*** .sub..epsilon. R molecule of the present invention can include chimeric molecules comprising a portion of a ***Fc*** .sub..epsilon. R molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the ***Fc*** .sub..epsilon. R portion binds to IgE in essentially the same manner as a ***Fc*** .sub..epsilon. R molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of. . .

SUMM A canine ***Fc*** .sub..epsilon. R molecule of the present invention can be contained in a formulation, herein referred to as a ***Fc*** .sub..epsilon. R formulation. For example, a canine ***Fc*** .sub..epsilon. R molecule can be combined with a buffer in which the

Fc .sub..epsilon. R is solubilized, and/or with a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a ***Fc*** .sub..epsilon. R can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate. . . are not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be combined with ***Fc*** .sub..epsilon. R or conjugated (i.e., attached) to ***Fc*** .sub..epsilon. R in such a manner as to not substantially interfere with the ability of the ***Fc*** .sub..epsilon. R to selectively bind to IgE.

SUMM A canine ***Fc*** .sub..epsilon. R molecule of the present invention can be bound to the surface of a cell expressing the ***Fc*** .sub..epsilon. R. A preferred ***Fc*** .sub..epsilon. R-bearing cell includes a recombinant cell expressing a nucleic acid molecule encoding a canine ***Fc*** .sub..epsilon. R alpha chain of the present invention. A more preferred recombinant cell of the present invention expresses a nucleic acid. . .

SUMM In addition, a ***Fc*** .sub..epsilon. R formulation of the present invention can include not only a ***Fc*** .sub..epsilon. R but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers. . .

SUMM The present invention also includes canine ***Fc*** .sub..epsilon. R mimetopes and use thereof to detect IgE. In accordance with the present invention, a "mimetope" refers to any compound that is able to mimic the ability of a canine ***Fc*** .sub..epsilon. R molecule to bind to canine IgE. A mimetope can be a peptide that has been modified to decrease its. . . by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of ***Fc*** .sub..epsilon. R mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex.RTM. technology, peptides identified by random screening of peptide libraries and proteins. . .

SUMM . . . the present invention is a method to detect canine IgE which includes the steps of: (a) contacting an isolated canine ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule with a putative canine IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon. R molecule:IgE complex; and (b) detecting levels of IgE by detecting said ***Fc*** .sub..epsilon. R molecule:IgE complex. Presence of such a ***Fc*** .sub..epsilon. R molecule:IgE complex indicates that the canine is producing IgE. The present method can further include the step of determining whether a canine IgE complexed with a canine ***Fc*** .sub..epsilon. R molecule is heat labile. Certain classes of IgE are heat labile when incubated at about 56.degree. C. for about. . . flea or heartworm allergens. Moreover, Applicants believe that identification of heat labile IgE and non-heat labile IgE using a canine ***Fc*** .sub..epsilon. R suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE. As such, a ***Fc*** .sub..epsilon. R molecule of the present invention may be useful for detecting molecules bound by the ***Fc*** .sub..epsilon. R molecule that are not identical to a known IgE.

SUMM . . . used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with a canine ***Fc*** .sub..epsilon. R molecule. Formation of a complex between a canine ***Fc*** .sub..epsilon. R and a canine IgE refers to the ability of the ***Fc*** .sub..epsilon. R to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a ***Fc*** .sub..epsilon. R of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between a ***Fc*** .sub..epsilon. R and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures,. . .

SUMM . . . are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between canine ***Fc*** .sub..epsilon. R and canine IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in. . .

SUMM . . . visually (e.g., either by eye or by a machine, such as a

densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the ***Fc*** .sub..epsilon. R or to a reagent that selectively binds to the ***Fc*** .sub..epsilon. R or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable. . . biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin.RTM.). Preferably, biotin is conjugated to an alpha chain of a ***Fc*** .sub..epsilon. R. Preferably a carbohydrate group of the ***Fc*** .sub..epsilon. R alpha chain is conjugated to biotin.

SUMM In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a canine ***Fc*** .sub..epsilon. R molecule that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a ***Fc*** .sub..epsilon. R molecule includes, but is not limited to, a radioactive label, a fluorescent label, a chemiluminescent label or a chromophoric label. A ***detectable*** ***marker*** is conjugated to a ***Fc*** .sub..epsilon. R molecule or a reagent in such a manner as not to block the ability of the ***Fc*** .sub..epsilon. R or reagent to bind to the IgE being detected. Preferably, a carbohydrate group of a ***Fc*** .sub..epsilon. R is conjugated to biotin.

SUMM In another embodiment, a ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting a putative IgE-containing composition with a ***Fc*** .sub..epsilon. R molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the ***Fc*** .sub..epsilon. R molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, a ***Fc*** .sub..epsilon. R molecule, an antigen, an antibody and a lectin, depending upon which portion of the ***Fc*** .sub..epsilon. R molecule:IgE complex is being detected. Preferred identifying labeled compounds that are antibodies include, for example, anti-IgE antibodies and anti- ***Fc*** .sub..epsilon. R antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a ***Fc*** .sub..epsilon. R molecule of the present invention produced in insect cells. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

SUMM In one preferred embodiment, a ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to a ***Fc*** .sub..epsilon. R molecule of the present invention. Examples of such a reagent include, but are not limited to, an antibody that selectively binds to a ***Fc*** .sub..epsilon. R molecule (referred to herein as an anti- ***Fc*** .sub..epsilon. R antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to a ***Fc*** .sub..epsilon. R molecule. ***Fc*** .sub..epsilon. R molecules conjugated to biotin are preferably detected using streptavidin, more preferably using ImmunoPure.RTM. NeutrAvidin.RTM. (available from Pierce, Rockford, Ill.).

SUMM In another preferred embodiment, a ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to an IgE antibody (referred. . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., an ***Fc*** receptor), and an antibody-binding complement protein. Preferred anti-IgE reagents include, but are not limited to, D9 (provided by Doug DeBoer,. . .

SUMM . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

SUMM A preferred immunoabsorbent assay method includes a step of either: (a) binding a canine ***Fc*** .sub..epsilon. R molecule to a substrate prior to contacting a canine ***Fc*** .sub..epsilon. R molecule with a putative IgE-containing composition to form a canine ***Fc*** .sub..epsilon. R molecule-coated substrate; or (b) binding a putative canine IgE-containing composition to a substrate prior to contacting a

canine ***Fc*** .sub..epsilon. R molecule with a putative IgE-containing composition to form a putative IgE-containing composition-coated substrate. Preferably, the substrate is a non-coated. . .

SUMM . . . whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, a canine ***Fc*** .sub..epsilon. R molecule of the present invention is used as a capture molecule when the ***Fc*** .sub..epsilon. R molecule is bound to a substrate. Alternatively, a canine ***Fc*** .sub..epsilon. R molecule is used as an indicator molecule when the ***Fc*** .sub..epsilon. R molecule is not bound to a substrate. Suitable molecules for use as capture molecules or indicator molecules include, but are not limited to, a canine ***Fc*** .sub..epsilon. R molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

SUMM . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

SUMM . . . formation of a complex between the indicator molecule and the antigen:IgE complex. The indicator molecule can be conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . . the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is a canine ***Fc*** .sub..epsilon. R molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

SUMM In one embodiment, a canine ***Fc*** .sub..epsilon. R molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well. . . A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for ***Fc*** .sub..epsilon. R molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain ***Fc*** .sub..epsilon. R molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the ***Fc*** .sub..epsilon. R is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the ***Fc*** .sub..epsilon. R molecule:IgE complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** , preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . .

SUMM . . . if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. A canine ***Fc*** .sub..epsilon. R molecule is added to the substrate and incubated to allow formation of a complex between the canine ***Fc*** .sub..epsilon. R molecule and the anti-IgE antibody:IgE complex. Preferably, the canine ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess ***Fc*** .sub..epsilon. R molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device. . .

SUMM . . . non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A canine ***Fc*** .sub..epsilon. R molecule is added to the substrate and incubated to allow formation of a complex between the canine ***Fc*** .sub..epsilon. R molecule and canine IgE. Preferably, the ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess ***Fc*** .sub..epsilon. R molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device. . .

SUMM . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . . labeling zone which is directed

downstream by the flow path. The capture zone contains the capture reagent, preferably a canine ***Fc*** .sub..epsilon. R molecule of the present invention that immobilizes canine IgE complexed to the antigen in the capture zone. The capture. . .

SUMM . . . to detect canine IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a canine ***Fc*** .sub..epsilon. R molecule of the present invention, the labeling reagent impregnated within the support structure in a labeling zone; and (c). . .

SUMM . . . the presence of canine IgE in a putative canine IgE-containing composition is determined by adding such composition to a canine ***Fc*** .sub..epsilon. R molecule of the present invention and an isolated canine IgE known to bind to the ***Fc*** .sub..epsilon. R molecule. The absence of binding of the ***Fc*** .sub..epsilon. R molecule to the known IgE indicating the presence of IgE in the putative IgE-containing composition.

SUMM . . . based, for example, on the disclosed detection methods. One embodiment is a kit to detect canine IgE comprising a canine ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and a means for detecting a canine IgE. Suitable and preferred canine ***Fc*** .sub..epsilon. R molecules are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the canine ***Fc*** .sub..epsilon. R molecule or to a canine IgE. A preferred kit of the present invention further comprises a detection means including. . . capable of selectively binding to canine IgE such as those disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a canine ***Fc*** .sub..epsilon. R molecule (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin).

SUMM . . . invention is a general allergen kit comprising an allergen common to all regions of the United States and a canine ***Fc*** .sub..epsilon. R molecule of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that. . .

SUMM . . . cod, halibut or and tuna, egg, milk, Brewer's yeast, whole wheat, corn, soybean, cheese and/or rice, and (b) a canine ***Fc*** .sub..epsilon. R molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

DETD This example describes the construction of recombinant baculoviruses expressing a truncated portion of the .alpha. chain of canine ***Fc*** .sub..epsilon. receptor.

DETD . . . molecules pVL-ncFc.sub..epsilon. R.alpha.1.sub.609, pVL-ncFc.sub..epsilon. R.alpha.2.sub.609, and pVL-ncFc.sub..epsilon. R.alpha.3.sub.617, each containing nucleic acid molecules encoding the extracellular domain of the canine ***Fc*** .sub..epsilon. R .alpha. chain, operatively linked to baculovirus polyhedron transcription control sequences were produced in the following manner. Three different canine ***Fc*** .sub..epsilon. R .alpha. chain extracellular domain nucleic acid molecule-containing fragments, each of about 608 to about 609 nucleotides were amplified by. . . ncFc.sub..epsilon. R.alpha.3.sub.617 each contained an about 608 to an about 609 nucleotide fragment encoding the extracellular domain of the canine ***Fc*** .sub..epsilon. R .alpha. chain, the coding strands of which have nucleic acid sequences denoted SEQ ID NO: 3, SEQ ID NO:. . .

DETD Translation of SEQ ID NO: 3 indicates that nucleic acid molecule ncFc.sub..epsilon. R.alpha.1.sub.609 encodes a ***Fc*** .sub..epsilon. R protein of about 197 amino acids, referred to herein as PcFc.sub..epsilon. R.alpha.1.sub.197, having amino acid sequence SEQ ID NO:. . .

DETD Translation of SEQ ID NO: 8 indicates that nucleic acid molecule ncFc.sub..epsilon. R.alpha.2.sub.609 encodes a ***Fc*** .sub..epsilon. R protein of about 197 amino acids, referred to herein as PcFc.sub..epsilon. R.alpha.2.sub.197, having amino acid sequence SEQ ID NO:. . .

DETD Translation of SEQ ID NO: 13 indicates that nucleic acid molecule ncFc.sub..epsilon. R.alpha.3.sub.617 encodes a ***Fc*** .sub..epsilon. R protein of about 199 amino acids, referred to herein as PcFc.sub..epsilon. R.alpha.3.sub.199, having amino acid sequence SEQ ID NO:. . .

DETD This example describes the production of PcFc.sub..epsilon. R.alpha.1.sub.197, PcFc.sub..epsilon. R.alpha.2.sub.197, and

PcFc.sub..epsilon. R.alpha.3.sub.199 canine ***Fc*** .sub..epsilon. R .alpha. chain proteins.

DETD . . . 18; "X" represents any amino acid). Comparison of SEQ ID NO: 18 and the amino acid sequence of the canine ***Fc*** .sub..epsilon. R alpha chain reported in Hayashi et al., ibid., indicated that PcFc.sub..epsilon. R.alpha.1.sub.197, PcFc.sub..epsilon. R.alpha.2.sub.197, and PcFc.sub..epsilon. R.alpha.3.sub.199, expressed in. . .

DETD This example describes the isolation, by DNA hybridization, and sequencing of a nucleic acid molecule encoding the ***Fc*** .sub..epsilon. R .alpha. chain from Canis canis.

DETD . . . library by the molecule's ability to hybridize with a .sup.32 P-labeled probe derived from a PCR clone encoding the canine ***Fc*** .sub..epsilon. R .alpha. chain. The canine mast cell cDNA library was prepared using standard techniques. Using a modification of the protocol. . . probe comprising ncFc.sub..epsilon. R.alpha.1.sub.609 (SEQ ID NO: 3). A plaque purified clone containing a canine nucleic acid molecule encoding the ***Fc*** .sub..epsilon. R .alpha. chain was converted into a double stranded recombinant molecule, using the ExAssist.TM. helper phage and SOLR.TM. E. coli. . . prepared using an alkaline lysis protocol, such as that described in Sambrook et al., ibid. The plasmid comprised a canine ***Fc*** .sub..epsilon. R .alpha. chain nucleic acid molecule of about 991 nucleotides denoted herein as ncFc.sub..epsilon. R.alpha.4.sub.991.

DETD . . . ID NO: 19. Translation of SEQ ID NO: 19 suggests that nucleic acid molecule ncFc.sub..epsilon. R.alpha.4.sub.991 encodes a full-length canine ***Fc*** .sub..epsilon. R .alpha. chain protein of about 253 amino acids, referred to herein as PcFc.sub..epsilon. R.alpha.4.sub.253, having amino acid sequence SEQ. . .

DETD . . . ID NO: 20 showed the most homology, i.e., about 100% identity between SEQ ID NO: 20 and a Canis canis ***Fc*** .sub..epsilon. R .alpha. chain protein (GenBank accession number D16413). Comparison of amino acid sequence SEQ ID NO: 22 with nucleic acid. . . NO: 22 showed the most homology, i.e., about 100% identity between SEQ ID NO: 22 and a canine mRNA for ***Fc*** .sub..epsilon. R .alpha. chain (GenBank accession D16413).

DETD This Example demonstrates the production of secreted canine ***Fc*** .sub..epsilon. R .alpha. chain protein in eukaryotic cells.

DETD To produce a secreted form of a canine ***Fc*** .sub..epsilon. R .alpha. chain, recombinant molecule pVL-ncFc.sub..epsilon. R.alpha.4.sub.591, containing a canine ***Fc*** .sub..epsilon. R .alpha. chain nucleic acid molecule encoding a secreted form of canine ***Fc*** .sub..epsilon. R .alpha. chain spanning nucleotides from about 35 through about 625 of SEQ ID NO: 19 operatively linked to baculovirus polyhedron transcription control sequences, was produced in the following manner. A canine ***Fc*** .sub..epsilon. R .alpha. chain nucleic acid molecule of about 591 nucleotides was PCR amplified from ncFc.sub..epsilon. R.alpha.4.sub.991 DNA using a sense. . .

DETD . . . by SEQ ID NO: 29. Translation of SEQ ID NO: 27 indicates that nucleic acid molecule ncFc.sub..epsilon. R.alpha.4.sub.591 encodes a ***Fc*** .sub..epsilon. R .alpha. chain protein of about 197 amino acids, referred to herein as PcFc.sub..epsilon. R.alpha.4.sub.197, having amino acid sequence SEQ ID NO: 28. Nucleic acid molecule ncFc.sub..epsilon. R.alpha..sub.591 encodes a secretable form of the canine ***Fc*** .sub..epsilon. R .alpha. chain. The processed protein product encoded by ncFc.sub..epsilon. R.alpha.4.sub.591 does not possess a leader sequence or transmembrane domain,. . .

DETD S. frugiperda:pVL-ncFc.sub..epsilon. R.alpha..sub.591 cells were cultured in order to produce a secreted canine ***Fc*** .sub..epsilon. R .alpha. chain protein, PcFc.sub..epsilon. R.alpha.4.sub.197 in the following manner. An about 1.5 liter cultures of serum-free ex-Cell Medium was. . .

CLM What is claimed is:
1. A method to detect canine IgE comprising: (a) contacting an isolated canine ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule with a putative canine IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon. R molecule:IgE complex; and (b) determining the presence of IgE by detecting said ***Fc*** .sub..epsilon. R molecule:IgE complex, the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex indicating the presence of IgE, wherein said canine ***Fc***

.sub..epsilon. R molecule comprises a protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 4,. . .

2. The method of claim 1, wherein said canine ***Fc*** .epsilon.R molecule is encoded by a nucleic acid molecule selected from the group consisting of ncFc.epsilon.R.alpha.1.sub.609, ncFc.epsilon.R.alpha.1.sub.591, ncFc.epsilon.R.alpha.2.sub.609, ncFc.epsilon.R.alpha.2.sub.591, ncFc.epsilon.R.alpha.3.sub.617, ncFc.epsilon.R.alpha.3.sub.597,. . .

3. The method of claim 1, wherein said canine ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of: (a) a nucleic acid molecule. . .

4. The method of claim 1, wherein said canine ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** .

5. The method of claim 1, wherein said canine ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a. . .

. . . 7. The method of claim 1 further comprising the step selected from the group consisting of: (a) immobilizing said canine ***Fc*** .sub..epsilon. R molecule to a substrate prior to performing stop (a) to form a ***Fc*** .sub..epsilon. R molecule-coated substrate; and (b) immobilizing said putative IgE-containing composition to a substrate prior to performing step (a) to form. . .

12. The method of claim 1, wherein said step of detecting comprises: (a) contacting said canine ***Fc*** .sub..epsilon. R molecule:IgE complex with an indicator molecule that binds selectively to said ***Fc*** .sub..epsilon. R molecule:IgE complex; (b) removing excess amounts of said indicator molecule that do not selectively bind to said ***Fc*** .sub..epsilon. R molecule:IgE complex; and (c) detecting said indicator molecule, wherein presence of said indicator molecule is indicative of the presence. . .

. . . 13. The method of claim 12, wherein said indicator molecule comprises a compound selected from the group consisting of a ***Fc*** .sub..epsilon. R molecule, an antigen, an antibody and a lectin.

14. The method of claim 1, said method comprising the steps of: (a) immobilizing said canine ***Fc*** .sub..epsilon. R molecule on a substrate; (b) contacting said canine ***Fc*** .sub..epsilon. R molecule with said putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon. R molecule:IgE complex bound to said substrate; (c) removing unbound material from said substrate under conditions that retain said ***Fc*** .sub..epsilon. R molecule:IgE complex binding to said substrate; and (d) detecting the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex.

15. The method of claim 14, wherein the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting said ***Fc*** .sub..epsilon. R molecule:IgE complex with a compound selected from the group consisting of an antigen and an antibody that binds selectively. . .

16. The method of claim 14, wherein said compound comprises a ***detectable*** ***marker*** .

17. The method of claim 1, said method comprising the steps of: (a) immobilizing a desired antigen on a substrate;. . . to said substrate; and (d) detecting the presence of said antigen:IgE complex by contacting said antigen:IgE complex with said canine ***Fc*** .sub..epsilon. R molecule.

. . . to said substrate; and (d) detecting the presence of said antibody:IgE complex by contacting said antibody:IgE complex with said canine ***Fc*** .sub..epsilon. R molecule.

. . . comprising the steps of: (a) immobilizing said putative IgE-containing composition on a substrate; (b) contacting said composition with said canine ***Fc*** .sub..epsilon. R molecule under conditions suitable for formation of a ***Fc*** .sub..epsilon. R molecule:IgE complex bound to said substrate; (e) removing unbound material from said substrate under conditions that retain said ***Fc*** .sub..epsilon. R molecule:IgE complex binding to said

substrate; and (d) detecting the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex.

20. The method of claim 19, wherein said canine ***Fc*** .sub..epsilon. R molecule comprises a ***detectable*** ***marker*** .

21. A kit for detecting canine IgE comprising a canine ***Fc*** .sub..epsilon. R molecule having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO:. . .
30. The kit of claim 21, wherein said detection means detects said canine ***Fc*** .sub..epsilon. R molecule.

31. The kit of claim 21, wherein said canine ***Fc*** .epsilon.R molecule is encoded by a nucleic acid molecule selected from the group consisting of ncFc.epsilon.R.alpha.1.sub.609, ncFc.epsilon.R.alpha.1.sub.591, ncFc.epsilon.R.alpha.2.sub.609, ncFc.epsilon.R.alpha.2.sub.591, ncFc.epsilon.R.alpha.3.sub.617, ncFc.epsilon.R.alpha.3.sub.597,. . .
32. The kit of claim 21, wherein said canine ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising. . .
33. The kit of claim 21, wherein said canine ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** .

34. The kit of claim 21, wherein said canine ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a. . .
. . . said labeling reagent is impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising said ***Fc*** .sub..epsilon. R molecule, wherein said capture reagent is located downstream of said labeling reagent within a capture zone fluidly connected to. . .

L13 ANSWER 22 OF 24 USPATFULL on STN
AN 2000:53911 USPATFULL
TI Equine ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules and uses thereof
IN Weber, Eric R., Fort Collins, CO, United States
McCall, Catherine A., Boulder, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 6057127 20000502
AI US 1998-15734 19980129 (9)
DT Utility
FS Granted
EXNAM Primary Examiner: Mertz, Prema; Assistant Examiner: Hamud, Fozia
LREP Heska Corporation
CLMN Number of Claims: 16
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2783
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to equine ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect IgE using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.
TI Equine ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules and uses thereof
AB The present invention relates to equine ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of. . . such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate ***Fc*** ***epsilon***

receptor -mediated biological responses.

SUMM The present invention relates to equine ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of. . .

SUMM Immunological stimulation can be mediated by IgE antibodies when IgE complexes with ***Fc*** ***epsilon*** ***receptors*** . ***Fc*** ***epsilon*** ***receptors*** are found on the surface of certain cell types, such as mast cells. Mast cells store biological mediators including histamine,. . .

SUMM The discovery of the present invention includes a novel equine ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..di-elect cons. R) alpha chain protein and the use of such a protein to detect the presence of IgE in a putative IgE-containing composition; to identify inhibitors of biological responses mediated by an equine ***Fc*** .sub..di-elect cons. R protein; and as a therapeutic compound to prevent or treat clinical symptoms that result from equine ***Fc*** .sub..di-elect cons. R-mediated biological responses.

SUMM Prior investigators have disclosed the nucleic acid sequence for: the ***human*** ***Fc*** .sub..di-elect cons. R alpha chain (Kochan et al., Nucleic Acids Res. 16:3584, 1988; Shimizu et al., Proc. Natl. Acad. Sci. USA 85:1907-1911, 1988; and Pang et al., J. Immunol. 151:6166-6174, 1993); the ***human*** ***Fc*** .sub..di-elect cons. R beta chain (Kuster et al., J. Biol. Chem. 267:12782-12787, 1992); the ***human*** ***Fc*** .sub..di-elect cons. R gamma chain (Kuster et al., J. Biol. Chem. 265:6448-6452, 1990); and the canine ***Fc*** .sub..di-elect cons. R alpha chain (GenBank.TM. accession number D16413). Although the subunits of ***human*** ***Fc*** .sub..di-elect cons. R have been known as early as 1988, they have never been used to identify an equine ***Fc*** .sub..di-elect cons. R. Similarly, even though the canine ***Fc*** .sub..di-elect cons. R chain has been known since 1993, it has never been used to identify an equine ***Fc*** .sub..di-elect cons. R. Moreover, the determination of ***human*** and canine ***Fc*** ***epsilon*** ***receptor*** sequences does not indicate, suggest or predict the cloning of a novel ***Fc*** .sub..di-elect cons. R gene from a different species, in particular, from an equine species. Previous investigators have found a low degree of similarity between rat, mouse and ***human*** ***Fc*** .sub..di-elect cons. R.alpha. (Ravtech et al., Ann. Rev. Immunol. Vol. 9, pp. 457-492, 1991). Thus, given this low degree of sequence similarity, it would appear only "obvious to try" to obtain an equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecule and protein.

SUMM . . . invention are needed in the art that will provide specific detection of IgE, in particular equine IgE, and treatment of ***Fc*** ***epsilon*** ***receptor*** -mediated disease.

SUMM The present invention relates to a novel product and process for detecting IgE and protecting animals from ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. According to the present invention there are provided equine ***Fc*** .sub..di-elect cons. R proteins and mimetopes thereof; equine ***Fc*** .sub..di-elect cons. R nucleic acid molecules, including those that encode such proteins; antibodies raised against such equine ***FcεR*** proteins (i.e., anti-equine ***Fc*** .sub..di-elect cons. R antibodies); and other compounds that inhibit the ability of equine ***Fc*** .sub..di-elect cons. R protein to form a complex with IgE (i.e, inhibitory compounds or inhibitors).

SUMM . . . comprising such proteins, mimetopes, nucleic acid molecules, antibodies, and/or inhibitory compounds, as well as use of such therapeutic compositions to ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

SUMM One embodiment of the present invention is an isolated nucleic acid molecule encoding an equine ***Fc*** .sub..di-elect cons. R protein. The equine ***Fc*** .sub..di-elect cons. R protein preferably includes: proteins comprising amino acid sequences SEQ ID NO:2, SEQ ID NO:7 and SEQ ID NO:. . . by allelic variants of nucleic acid molecules encoding a protein comprising any of the amino acid sequences. Particularly preferred equine ***Fc*** .sub..di-elect cons. R nucleic acid molecules include: nucleic acid molecules comprising nucleic acid sequences SEQ ID NO: 1, SEQ ID NO:3,. . .

SUMM The present invention also includes an isolated equine ***Fc***

.sub..di-elect cons. R protein. A preferred equine ***Fc*** .sub..di-elect cons. R protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a nucleic acid sequence including SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:8. Particularly preferred equine ***Fc*** .sub..di-elect cons. R proteins include at least one of the following amino acid sequences: SEQ ID NO:2, SEQ ID NO:7 and. . . .

SUMM The present invention also relates to recombinant molecules, recombinant viruses and recombinant cells that include equine ***Fc*** .sub..di-elect cons. R nucleic acid molecules of the present invention. Also included are methods to produce such nucleic acid molecules, recombinant. . . .

SUMM . . . detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated equine ***Fc*** .sub..di-elect cons. R molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..di-elect cons. R molecule:IgE complex; and (b) determining the presence of IgE by detecting the ***Fc*** .sub..di-elect cons. R molecule:IgE complex, the presence of the ***Fc*** .sub..di-elect cons. R molecule:IgE complex indicating the presence of IgE. A preferred equine ***Fc*** .sub..di-elect cons. R molecule is one in which a carbohydrate group of the equine ***FCER*** molecule is conjugated to biotin.

SUMM . . . IgE-containing composition under conditions suitable for formation of a recombinant cell:IgE complex, in which the recombinant cell comprises an equine ***Fc*** .sub..di-elect cons. R molecule; and (b) determining the presence of IgE by detecting the recombinant cell:IgE complex, the presence of the recombinant cell:IgE complex indicating the presence of IgE. A preferred method to detect IgE comprises: (a) immobilizing the ***Fc*** .sub..di-elect cons. R molecule on a substrate; (b) contacting the ***Fc*** .sub..di-elect cons. R molecule with the putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..di-elect cons. R molecule:IgE complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain ***Fc*** .sub..di-elect cons. R molecule:IgE complex binding to the substrate; and (d) detecting the presence of the ***Fc*** .sub..di-elect cons. R molecule:IgE complex. Another preferred method to detect IgE comprises: (a) immobilizing a specific antigen on a substrate; (b). . . binding to said substrate; and (d) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with said ***Fc*** .sub..di-elect cons. R molecule. Another preferred method to detect IgE comprises: (a) immobilizing an antibody that binds selectively to IgE on. . . binding to the substrate; and (d) detecting the presence of the antibody:IgE complex by contacting the antibody:IgE complex with said ***Fc*** .sub..di-elect cons. R molecule. Another preferred method to detect IgE comprises: (a) immobilizing a putative IgE-containing composition on a substrate; (b) contacting the composition with the ***Fc*** .sub..di-elect cons. R molecule under conditions suitable for formation of a ***Fc*** .sub..di-elect cons. R molecule:IgE complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain ***Fc*** .sub..di-elect cons. R molecule:IgE complex binding to the substrate; and (d) detecting the presence of the ***Fc*** .sub..di-elect cons. R molecule:IgE complex.

SUMM . . . a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising an equine ***Fc*** .sub..di-elect cons. R protein and a means for detecting IgE.

SUMM The present invention also includes an inhibitor that interferes with formation of a complex between equine ***Fc*** .sub..di-elect cons. R protein and IgE, in which the inhibitor is identified by its ability to interfere with the complex formation. A particularly preferred inhibitor includes a substrate analog of an equine ***Fc*** .sub..di-elect cons. R protein, a mimetope of an equine ***Fc*** .sub..di-elect cons. R protein and a soluble portion of an equine ***Fc*** .sub..di-elect cons. R protein. Also included is a method to identify a compound that interferes with formation of a complex between equine ***Fc*** .sub..di-elect cons. R protein and IgE, the method comprising: (a) contacting an isolated equine ***Fc*** .sub..di-elect cons. R protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the equine ***Fc*** .sub..di-elect

cons. R protein forms a complex with IgE; and (b) determining if the putative inhibitory compound inhibits the complex formation.. . . test kit is also included to identify a compound capable of interfering with formation of a complex between an equine ***Fc*** .sub..di-elect cons. R protein and IgE, the test kit comprising an isolated equine ***Fc*** .sub..di-elect cons. R protein that can complex with IgE and a means for determining the extent of interference of the complex. . .

SUMM Yet another embodiment of the present invention is a therapeutic composition that is capable of reducing ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. Such a therapeutic composition includes one or more of the following therapeutic compounds: an isolated equine ***Fc*** .sub..di-elect cons. R protein; a mimetope of an equine ***Fc*** .sub..di-elect cons. R protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with an equine ***Fc*** .sub..di-elect cons. R gene; an isolated antibody that selectively binds to an equine ***Fc*** .sub..di-elect cons. R protein; and an inhibitor that interferes with formation of a complex between an equine ***Fc*** .sub..di-elect cons. R protein and IgE. A method of the present invention includes the step of administering to an animal a. . .

SUMM Yet another embodiment of the present invention is a method to produce an equine ***Fc*** .sub..di-elect cons. R protein, the method comprising culturing a cell transformed with a nucleic acid molecule encoding an equine ***Fc*** .sub..di-elect cons. R protein.

SUMM The present invention provides for isolated equine ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .sub..di-elect cons. R.alpha.) proteins, isolated equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecules, antibodies directed against equine ***Fc*** .sub..di-elect cons. R.alpha. proteins and other inhibitors of equine ***Fc*** .sub..di-elect cons. R.alpha. activity. As used herein, the terms isolated equine ***Fc*** .sub..di-elect cons. R.alpha. proteins and isolated equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecules refers to ***Fc*** .sub..di-elect cons. R.alpha. proteins and ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecules derived from horses and, as such, can be obtained from their natural source or can. . . of the present invention are advantageous because they enable the detection of IgE and the inhibition of IgE or equine ***Fc*** .sub..di-elect cons. R.alpha. protein activity associated with disease. As used herein, equine ***Fc*** epsilon alpha chain receptor protein can be referred to as ***Fc*** .sub..di-elect cons. R.alpha. protein or ***Fc*** .sub..di-elect cons. R alpha chain protein.

SUMM One embodiment of the present invention is an isolated protein comprising an equine ***Fc*** .sub..di-elect cons. R.alpha. protein. It is to be noted that the term "a" or "an" entity refers to one or more. . .

SUMM As used herein, an isolated equine ***Fc*** .sub..di-elect cons. R.alpha. protein can be a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. Preferably, an equine ***Fc*** .sub..di-elect cons. R.alpha. protein comprises at least a portion of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein that binds to IgE, i.e., that is capable of forming a complex with an IgE.

SUMM An equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to bind to IgE. Examples of equine ***Fc*** .sub..di-elect cons. R.alpha. protein homologs include equine ***Fc*** .sub..di-elect cons. R.alpha. proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a. . .

SUMM Equine ***Fc*** .sub..di-elect cons. R.alpha. protein homologs can be the result of natural allelic variation or natural mutation. Equine ***Fc*** .sub..di-elect cons. R.alpha. protein homologs of the present invention can also be produced using techniques known in the art including, but. . .

SUMM Isolated equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention have the further characteristic of being encoded by nucleic acid molecules that hybridize under stringent hybridization conditions to a gene encoding an equine ***Fc*** .sub..di-elect cons. R.alpha. protein. As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid

molecules, including. . .

SUMM As used herein, an equine ***Fc*** .sub..di-elect cons. R.alpha. gene includes all nucleic acid sequences related to a natural equine ***Fc*** .sub..di-elect cons. R.alpha. gene such as regulatory regions that control production of the equine ***Fc*** .sub..di-elect cons. R.alpha. protein encoded by that gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. In one embodiment, an equine ***Fc*** .sub..di-elect cons. R.alpha. gene of the present invention includes nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4,. . .

SUMM . . . other nucleic acid and protein sequences presented herein) represent apparent nucleic acid sequences of certain nucleic acid molecules encoding equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention.

SUMM In another embodiment, an equine ***Fc*** .sub..di-elect cons. R.alpha. gene can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:1,. . . NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8 and/or SEQ ID NO:11. An allelic variant of an equine ***Fc*** .sub..di-elect cons. R.alpha. gene is a gene that occurs at essentially the same locus (or loci) in the genome as the. . .

SUMM The minimal size of a ***Fc*** .sub..di-elect cons. R.alpha. protein homolog of the present invention is a size sufficient to be encoded by a nucleic acid molecule. . . length if they are AT-rich. As such, the minimal size of a nucleic acid molecule used to encode an equine ***Fc*** .sub..di-elect cons. R.alpha. protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein homolog of the present invention is from about 4 to about 6 amino acids in length. There. . .

SUMM . . . used herein, an equine refers to any member of the horse family. Examples of horses from which to isolate equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention (including isolation of the natural protein or production of the protein by recombinant. . .

SUMM Suitable horse cells from which to isolate an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention include cells that have ***Fc*** .sub..di-elect cons. R.alpha. proteins. Preferred horse cells from which to obtain an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention include basophil cells, mast cells, mastocytoma cells, dendritic cells, B lymphocytes, macrophages, eosinophils, and/or monocytes. An equine ***Fc*** .sub..di-elect cons. R.alpha. of the present invention is preferably obtained from mastocytoma cells, mast cells or basophil cells.

SUMM The present invention also includes mimetopes of equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention. As used herein, a mimetope of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention refers to any compound that is able to mimic the activity of such an equine ***Fc*** .sub..di-elect cons. R.alpha. protein (e.g., ability to bind to IgE), often because the mimetope has a structure that mimics the equine ***Fc*** .sub..di-elect cons. R.alpha. protein. It is to be noted, however, that the mimetope need not have a structure similar to an equine ***Fc*** .sub..di-elect cons. R.alpha. protein as long as the mimetope functionally mimics the protein. Mimetopes can be, but are not limited to:. . . nucleic acids; and/or any other peptidomimetic compounds. Mimetopes of the present invention can be designed using computer-generated structures of equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as. . . other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., an equine IgE ***Fc*** domain or anti-equine ***Fc*** .sub..di-elect cons. R.alpha. antibody). A mimetope can also be obtained by, for example, rational drug design. In a rational drug design. . . for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of equine ***Fc*** .sub..di-elect cons. R.alpha. mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex.TM. technology, peptides identified by random screening of peptide libraries and. . . by phage display technology. A preferred mimetope is a peptidomimetic

compound that is structurally and/or functionally similar to an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention, particularly to the IgE ***Fc*** domain binding site of the equine ***Fc*** .sub..di-elect cons. R.alpha. protein. As used herein, the ***Fc*** domain of an antibody refers to the portion of an immunoglobulin that has ***Fc*** receptor binding effector function. Typically, the ***Fc*** domain of an IgE comprises the CH2 and CH3 domains of the heavy chain constant region.

SUMM According to the present invention, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule of the present invention refers to: an equine ***Fc*** .sub..di-elect cons. R.alpha. protein, in particular a soluble equine ***Fc*** .sub..di-elect cons. R.alpha. protein; an equine ***Fc*** .sub..di-elect cons. R.alpha. homolog; an equine ***Fc*** .sub..di-elect cons. R.alpha. mimetope; an equine ***Fc*** .sub..di-elect cons. R.alpha. substrate analog; or an equine ***Fc*** .sub..di-elect cons. R.alpha. peptide. Preferably, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule binds to IgE.

SUMM One embodiment of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention is a fusion protein that includes an equine ***Fc*** .sub..di-elect cons. R.alpha. protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention. . . to, segments that can: enhance a protein's stability; act as an immunopotentiator to enhance an immune response against an equine ***Fc*** .sub..di-elect cons. R.alpha. protein; and/or assist purification of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has. . . protein, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the equine ***Fc*** .sub..di-elect cons. R.alpha.-containing domain of the protein and can be susceptible to cleavage in order to enable straight-forward recovery of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid molecule. . . that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of an equine ***Fc*** .sub..di-elect cons. R.alpha.-containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; ***Fc*** receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); a "tag" domain (e.g., at. . . the domain, such as monoclonal antibodies); and/or a linker and enzyme domain (e.g., alkaline phosphatase domain connected to an equine ***Fc*** .sub..di-elect cons. R.alpha. protein by a linker). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a. . .

SUMM A preferred equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions. . . following nucleic acid molecules: neqFc.sub..di-elect cons. R.alpha..sub.1015, neqFc.sub..di-elect cons. R.alpha..sub.765, neqFc.sub..di-elect cons. R.alpha..sub.708 and neqFc.sub..di-elect cons. R.alpha..sub.603. Preferably, the equine ***Fc*** .sub..di-elect cons. R.alpha. protein binds to IgE. A further preferred isolated protein is encoded by a nucleic acid molecule that hybridizes. . .

SUMM . . . acid sequences reported in GenBank.TM. indicates that SEQ ID NO:2 showed the most homology, i.e., about 61% identity, with a ***human*** high affinity IgE receptor .alpha.-subunit (SwissProt accession number P12319).

SUMM More preferred equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention include proteins comprising amino acid sequences that are at least about 65%, preferably. . .

SUMM More preferred equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention include proteins encoded by a nucleic acid molecule comprising at least a portion. . . allelic variants of such nucleic acid molecules, the portion being capable of binding to IgE. More preferred is an equine ***Fc*** .sub..di-elect cons. R.alpha. protein encoded by neqFc.sub..di-elect cons. R.alpha..sub.1015, neqFc.sub..di-elect cons. R.alpha..sub.765, neqFc.sub..di-elect cons. R.alpha..sub.708 and/or neqFc.sub..di-elect cons. R.alpha..sub.603, or

by an allelic variant of such nucleic acid molecules. Particularly preferred equine ***Fc*** .sub..di-elect cons. R.alpha. proteins are PequFc.sub..di-elect cons. R.alpha..sub.255, PequFc.sub..di-elect cons. R.alpha..sub.236 and PequFc.sub..di-elect cons. R.alpha..sub.201.

SUMM In one embodiment, a preferred equine ***Fc*** .sub..di-elect cons. ROx protein of the present invention is encoded by at least a portion of SEQ ID NO:1, SEQ ID. . . .

SUMM Also preferred is an equine ***Fc*** .sub..di-elect cons. R.alpha. protein encoded by an allelic variant of a nucleic acid molecule comprising at least a portion of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:6 and/or SEQ ID NO:11. Particularly preferred equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention include SEQ ID NO:2, SEQ ID NO:7 and SEQ ID NO:12 (including, but. . .

SUMM . . . embodiment of the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with an equine ***Fc*** .sub..di-elect cons. R.alpha. gene. The identifying characteristics of such a gene are heretofore described. A nucleic acid molecule of the present invention can include an isolated natural equine ***Fc*** .sub..di-elect cons. R.alpha. gene or a homolog thereof, the latter of which is described in more detail below. A nucleic acid. . . nucleic acid molecule of the present invention is the minimal size that can form a stable hybrid with an equine ***Fc*** .sub..di-elect cons. R.alpha. gene under stringent hybridization conditions.

SUMM . . . molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to ***human*** manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecule of the present invention can be isolated from its natural source or can be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecules can include, for example, natural allelic variants and nucleic acid molecules modified by nucleotide insertions,. . . a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention or to form stable hybrids under stringent conditions with natural gene isolates.

SUMM An equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecule homolog can be produced using a number of methods known to those skilled in the. . . mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with an equine ***Fc*** .sub..di-elect cons. R.alpha. gene or by screening for function of a protein encoded by the nucleic acid molecule (e.g., ability of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein to bind equine IgE).

SUMM . . . isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule". . . interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding an equine ***Fc*** .sub..di-elect cons. R.alpha. protein.

SUMM One embodiment of the present invention is an equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecule that hybridizes under stringent hybridization conditions with nucleic acid molecule neqFc.sub..di-elect cons. R.alpha..sub.1015 and preferably. . .

SUMM . . . acid sequences reported in GenBank indicates that SEQ ID NO:1 showed the most homology, i.e., about 75% identity to a ***human*** mRNA for immunoglobulin E receptor alpha chain gene (Accession number X06948).

SUMM Preferred equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecules include nucleic acid molecules having a nucleic acid sequence that is at least about 80%,. . .

SUMM . . . ID NO:5, SEQ ID NO:6, SEQ ID NO:8 and/or SEQ ID NO:11, that is capable of hybridizing to an equine ***Fc*** .sub..di-elect cons. R.alpha. gene of the present invention, as well as allelic variants thereof. A more preferred nucleic acid molecule includes. . .

SUMM Preferred equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecules also include nucleic acid molecules having a nucleic acid sequence that is at least about. . .

SUMM Knowing the nucleic acid sequences of certain equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make. . . (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecules from other horses. Such nucleic acid molecules can be obtained in a variety of ways. . .

SUMM . . . conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising equine ***Fc*** .sub..di-elect cons. R.alpha. genes or other equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecules. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum. . . . for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules or therapeutic reagents to inhibit equine ***Fc*** .sub..di-elect cons. R.alpha. protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also. . .

SUMM . . . is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulation of equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecules of the present invention.

SUMM . . . the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain. . .

SUMM . . . that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecules disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include neqFc.sub..di-elect. . .

SUMM . . . production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention or can be capable of producing such proteins after being transformed with at. . . cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., ***human*** , murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK.sup.31 cells and/or. . .

SUMM Isolated equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention can be produced in a variety of ways, including production and recovery of. . . permit protein production. An effective medium refers to any medium in which a cell is cultured to produce an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention. Such a medium typically comprises an aqueous medium having assimilable carbon, nitrogen and. . .

SUMM The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention or a mimetope thereof (i.e., anti-equine ***Fc*** .sub..di-elect cons. R.alpha. antibodies). As used herein, the term "selectively binds to" an equine ***Fc*** .sub..di-elect cons. R.alpha. protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and. . . in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., ibid. An anti-equine ***Fc*** .sub..di-elect cons. R.alpha. antibody preferably selectively binds to an equine ***Fc*** .sub..di-elect cons. R.alpha. protein in such a way as to reduce the activity of that protein.

SUMM . . . antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies. . .

SUMM . . . are within the scope of the present invention. For example, such antibodies can be used (a) as tools to detect ***Fc*** ***epsilon*** ***receptor*** in the presence or absence of IgE and/or (b) as tools to screen expression libraries and/or to recover desired proteins. . . proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target cytotoxic agents to cells having ***Fc*** ***epsilon*** ***receptors*** such as those disclosed herein in order to directly kill such cells. Targeting can be accomplished by conjugating (i.e., stably. . . in the art. Suitable cytotoxic agents are known to those skilled in the art. Antibodies of the present invention, including ***Fc*** .sub..di-elect cons. R.alpha.-binding portions thereof, can also be used, for example, to inhibit binding of IgE to ***Fc*** ***epsilon*** ***receptors*** , to produce anti-equine ***Fc*** .sub..di-elect cons. R.alpha. idiotypic antibodies, to purify cells having equine ***Fc*** .sub..di-elect cons. R.alpha. proteins, to stimulate intracellular signal transduction through an equine ***Fc*** .sub..di-elect cons. R.alpha. and to identify cells having equine ***Fc*** .sub..di-elect cons. R.alpha. proteins.

SUMM An equine ***Fc*** .sub..di-elect cons. R.alpha. molecule of the present invention can include chimeric molecules comprising a portion of an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the ***Fc*** .sub..di-elect cons. R.alpha. molecule portion binds to IgE in essentially the same manner as a ***Fc*** .sub..di-elect cons. R.alpha. molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion. . .

SUMM An equine ***Fc*** .sub..di-elect cons. R.alpha. molecule of the present invention can include chimeric molecules comprising a portion of an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule that binds to an IgE and a second molecule, such as an enzyme, that enables the chimeric molecule to bind to IgE in essentially the same manner as a ***Fc*** .sub..di-elect cons. R.alpha. molecule which does not include such a second molecule, and to hydrolyze a substrate in such a manner. . . An example of a suitable second molecule includes alkaline phosphatase, horse radish peroxidase or urease. In one embodiment an equine ***Fc*** .sub..di-elect cons. R.alpha. chimeric molecule of the present invention comprises a protein encoded by a recombinant molecule comprising a nucleic acid molecule that encodes at least a portion of an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule that binds to an IgE, operatively linked to a nucleic acid molecule that encodes an enzyme, preferably. . .

SUMM An equine ***Fc*** .sub..di-elect cons. R.alpha. molecule of the present invention can be contained in a formulation, herein referred to as a ***Fc*** .sub..di-elect cons. R.alpha. molecule formulation. For example, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule can be combined with a buffer in which the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is solubilized, and/or with a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water,. . . not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be mixed with equine ***Fc*** .sub..di-elect cons. R.alpha. molecules or conjugated (i.e., attached) to equine ***Fc*** .sub..di-elect cons. R.alpha. molecules in such a manner as to not substantially interfere with the ability of the equine ***Fc*** .sub..di-elect cons. R.alpha. molecules to selectively bind to IgE.

SUMM An equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention can be bound to the surface of a cell comprising the equine ***Fc*** .sub..di-elect cons. R.alpha. protein. A preferred equine ***Fc*** .sub..di-elect cons. R.alpha. protein-bearing cell includes a recombinant cell comprising a nucleic acid molecule encoding an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention. A more preferred recombinant cell of the present invention comprises a nucleic acid. . .

SUMM In addition, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule formulation of the present invention can include not only an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule but also one

or more additional antigens or antibodies useful in detecting IgE. As used herein, an. . . .

SUMM . . . of the present invention is a method to detect IgE which includes the steps of: (a) contacting an isolated equine ***Fc*** .sub..di-elect cons. R.alpha. molecule with a putative IgE-containing composition under conditions suitable for formation of an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule:IgE complex; and (b) detecting the presence of IgE by detecting the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule:IgE complex. Presence of such an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule:IgE complex indicates that the animal is producing IgE. Preferred IgE to detect using an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule include equine IgE, canine IgE, feline IgE and ***human*** IgE, with equine IgE being particularly preferred. The present method can further include the step of determining whether an IgE complexed with an equine ***Fc*** .sub..di-elect cons. R.alpha. protein is heat labile. Preferably, a heat labile IgE is determined by incubating an IgE at about 56.degree.. . . .

SUMM . . . used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule. Formation of a complex between an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule and an IgE refers to the ability of the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations,. . . .

SUMM . . . are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between equine ***Fc*** .sub..di-elect cons. R.alpha. molecule and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods. . . .

SUMM . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule or to a reagent that selectively binds to the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples. . . compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin available from Pierce, Rockford, Ill.). According to the present invention, a ***detectable*** ***marker*** can be connected to an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule using, for example, chemical conjugation or recombinant DNA technology (e.g., connection of a fusion segment such as. . . binding domain; an immunoglobulin binding; a sugar binding domain; and a "tag" domain). Preferably a carbohydrate group of the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is chemically conjugated to biotin.

SUMM In one embodiment, a complex is detected by contacting a putative IgE-containing composition with an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule includes, but is not limited to, a radioactive label, a fluorescent label, an enzyme label, a chemiluminescent label, a chromophoric label or a ligand. A ***detectable*** ***marker*** is conjugated to an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule in such a manner as not to block the ability of the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule to bind to the IgE being detected. Preferably, a carbohydrate group of an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is conjugated to biotin.

SUMM In another embodiment, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule:IgE complex is detected by contacting a putative IgE-containing composition with an equine ***Fc*** .sub..di-elect

cons. R.alpha. molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, an antigen, an antibody and a lectin, depending upon which portion of the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule:IgE complex is being detected. Preferred indicator molecules that are antibodies include, for example, anti-IgE antibodies and anti-equine ***Fc*** .sub..di-elect cons. R.alpha. antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention produced in insect cells. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

SUMM In one preferred embodiment, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule:IgE complex is detected by contacting the complex with an indicator molecule that selectively binds to an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule of the present invention. Examples of such indicator molecule includes, but are not limited to, an antibody that selectively binds to an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule (referred to herein as an anti-equine ***Fc*** .sub..di-elect cons. R.alpha. antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule. An equine ***Fc*** .sub..di-elect cons. R.alpha. molecule conjugated to biotin is preferably detected using streptavidin.

SUMM In another preferred embodiment, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule:IgE complex is detected by contacting the complex with indicator molecule that selectively binds to an IgE antibody. . . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., a ***Fc*** receptor), and an antibody-binding complement protein. A preferred indicator molecule includes an anti-equine IgE antibody. As used herein, an anti-IgE. . .

SUMM . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

SUMM A preferred immunoabsorbent assay method includes a step of either: (a) immobilizing an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule on a substrate prior to contacting an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule with a putative IgE-containing composition to form an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule-immobilized substrate; and (b) binding a putative IgE-containing composition on a substrate prior to contacting an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule with a putative IgE-containing composition to form a putative IgE-containing composition-bound substrate. Preferably, the substrate includes a non-coated substrate, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule-immobilized substrate, an antigen-immobilized substrate or an anti-IgE antibody-immobilized substrate.

SUMM . . . whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule of the present invention is used as a capture molecule when the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is bound on a substrate. Alternatively, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is used as an indicator molecule when the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is not bound on a substrate. Suitable molecules for use as capture molecules or indicator molecules include, but are not limited to, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

SUMM . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary

molecules. . .

SUMM . . . the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

SUMM In one embodiment, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish. . . . biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for equine ***Fc*** .sub..di-elect cons. R.alpha. molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain equine ***Fc*** .sub..di-elect cons. R.alpha. molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule:IgE complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . .

SUMM . . . if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. An equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule and the anti-IgE antibody:IgE complex. Preferably, the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection. . .

SUMM . . . non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. An equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule and the IgE. Preferably, the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection. . .

SUMM . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . . which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture. . .

SUMM . . . used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c). . .

SUMM . . . in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule of the present invention and an isolated IgE known to bind to the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule. The absence of binding of the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule to the known IgE indicates the presence of IgE in the putative IgE-containing composition. The known IgE is preferably conjugated to a ***detectable*** ***marker*** .

SUMM . . . IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising an equine ***Fc*** .sub..di-elect cons. R.alpha. protein and a means for detecting an IgE. Suitable and preferred equine ***Fc*** .sub..di-elect cons. R.alpha.

protein are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the equine ***Fc*** .sub..di-elect cons. R.alpha. protein or to an IgE. A preferred kit of the present invention further comprises a detection means including. . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to an equine ***Fc*** .sub..di-elect cons. R.alpha. protein (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including equines, canines and/or felines.

SUMM . . . invention is a general allergen kit comprising an allergen common to all regions of the United States and an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens. . .

SUMM . . . allergen including wheat, corn, alfalfa, hay, straw, oats, grains, processed grain by-products and grasses and/or dusts thereof, and an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule of the present invention. Kits for detecting hypersensitivity to feeds and/or feed dust allergens can be used. . .

SUMM . . . present invention is a therapeutic composition that, when administered to an animal in an effective manner, is capable of reducing ***Fc*** receptor mediated reactions associated with diseases related to biological responses involving ***Fc*** receptor function. A therapeutic composition of the present invention can include: an isolated equine ***Fc*** .sub..di-elect cons. R.alpha. protein, or homolog thereof; a mimetope of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with an equine ***Fc*** .sub..di-elect cons. R.alpha. gene; an isolated antibody that selectively binds to an equine ***Fc*** .sub..di-elect cons. R.alpha. protein; and/or an inhibitor that interferes with formation of a complex between an equine ***Fc*** .sub..di-elect cons. R.alpha. protein and IgE.

SUMM One embodiment of a therapeutic composition of the present invention is a therapeutic compound comprising an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule of the present invention, that binds to an IgE. According to the present invention, an equine ***Fc*** .sub..di-elect cons. R.alpha. molecule competes for IgE with naturally-occurring ***Fc*** ***epsilon*** ***receptors*** , particularly those on mastocytoma cells, mast cells or basophils, so that IgE is bound to the administered equine ***Fc*** .sub..di-elect cons. R.alpha. molecule and thus is unable to bind to ***Fc*** ***epsilon*** ***receptor*** on a cell, thereby inhibiting mediation of a biological response. Preferred equine ***Fc*** .sub..di-elect cons. R.alpha. molecule for use in a therapeutic composition comprises an equine ***Fc*** .sub..di-elect cons. R.alpha. protein, or homolog thereof, as described herein, particularly a fragment thereof, which binds to IgE. Equine ***Fc*** .sub..di-elect cons. R.alpha. molecules for use in a therapeutic composition can be in a monovalent and/or multivalent form, so long as the equine ***Fc*** .sub..di-elect cons. R.alpha. molecule is capable of binding to IgE. A more preferred equine ***Fc*** .sub..di-elect cons. R.alpha. molecule for use in a therapeutic composition includes a soluble fragment of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein. A preferred equine ***Fc*** .sub..di-elect cons. R.alpha. protein is encoded by neqFc.sub..di-elect cons. RC.sub.603 and an even more preferred equine ***Fc*** .sub..di-elect cons. R.alpha. protein is PequFc.sub..di-elect cons. R.alpha..sub.201.

SUMM . . . therapeutic composition of the present invention comprises a therapeutic compound that interferes with the formation of a complex between equine ***Fc*** .sub..di-elect cons. R.alpha. protein and IgE, usually by binding to or otherwise interacting with or otherwise modifying the equine ***Fc*** .sub..di-elect cons. R.alpha. protein's IgE binding site. Equine ***Fc*** .sub..di-elect cons. R.alpha. protein inhibitors can also interact with other regions of the equine ***Fc*** .sub..di-elect cons. R.alpha. protein to inhibit equine ***Fc*** .sub..di-elect cons. R.alpha. protein activity, for example, by allosteric interaction. An inhibitor of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein can interfere with ***Fc***

.sub..di-elect cons. R.alpha. protein and IgE complex formation by, for example, preventing formation of a ***Fc*** .sub..di-elect cons. R.alpha. protein and IgE complex or disrupting an existing ***Fc*** .sub..di-elect cons. R.alpha. protein and IgE complex causing the ***Fc*** .sub..di-elect cons. R.alpha. protein and IgE to dissociate. An inhibitor of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein is usually a relatively small molecule. Preferably, an equine ***Fc*** .sub..di-elect cons. R.alpha. protein inhibitor of the present invention is identified by its ability to bind to, or otherwise interact with, an equine ***Fc*** .sub..di-elect cons. R.alpha. protein, thereby interfering with the formation of a complex between an equine ***Fc*** .sub..di-elect cons. R.alpha. protein and IgE.

SUMM Preferred inhibitors of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention include, but are not limited to, a substrate analog of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein, a mimetope of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein, a soluble (i.e., secreted form of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein) portion of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein that binds to IgE, and other molecules that bind to an equine ***Fc*** .sub..di-elect cons. R.alpha. protein (e.g., to an allosteric site) in such a manner that IgE-binding activity of the equine ***Fc*** .sub..di-elect cons. R.alpha. protein is inhibited. An equine ***Fc*** .sub..di-elect cons. R.alpha. protein substrate analog refers to a compound that interacts with (e.g., binds to, associates with, modifies) the IgE-binding site of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein. A preferred equine ***Fc*** .sub..di-elect cons. R.alpha. protein substrate analog inhibits IgE-binding activity of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein. Equine ***Fc*** .sub..di-elect cons. R.alpha. protein substrate analogs can be of any inorganic or organic composition, and, as such, can be, but are not limited to, peptides, nucleic acids, and peptidomimetic compounds. Equine ***Fc*** .sub..di-elect cons. R.alpha. protein substrate analogs can be, but need not be, structurally similar to an equine ***Fc*** .sub..di-elect cons. R.alpha. protein's natural substrate (e.g., IgE) as long as they can interact with the active site (e.g., IgE-binding site of that equine ***Fc*** .sub..di-elect cons. R.alpha.). Equine ***Fc*** .sub..di-elect cons. R.alpha. protein substrate analogs can be designed using computer-generated structures of equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention or computer structures of, for example, the ***Fc*** domain of IgE. Substrate analogs can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides, peptidomimetic. . . inorganic or organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., an equine ***FcεRa*** protein or anti-equine ***Fc*** .sub..di-elect cons. R.alpha. idiotypic antibody). A preferred equine ***Fc*** .sub..di-elect cons. R.alpha. protein substrate analog is a peptidomimetic compound (i.e., a compound that is structurally and/or functionally similar to a natural substrate of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein of the present invention, particularly to the region of the substrate that binds to an equine ***Fc*** .sub..di-elect cons. R.alpha. protein, but that inhibits IgE binding upon interacting with the IgE binding site).

SUMM Equine ***Fc*** .sub..di-elect cons. R.alpha. molecules, as well as other inhibitors and therapeutic compounds, can be used directly as compounds in compositions of. . .

SUMM In one embodiment, a therapeutic composition of the present invention can be used to reduce a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response in an animal by administering such a composition to an animal. Preferably, an animal is treated by administering. . . therapeutic composition of the present invention in such a manner that a therapeutic compound (e.g., an inhibitor of an equine ***Fc*** .sub..di-elect cons. R.alpha. protein, an anti-equine ***Fc*** .sub..di-elect cons. R.alpha. antibody, an inhibitor of IgE, or nucleic acid molecules encoding equine ***Fc*** .sub..di-elect cons. R.alpha. proteins) binds to an IgE or a ***Fc*** ***epsilon*** ***receptor*** in the animal. Such administration could be by a variety of routes known to those skilled in the art including,. . .

SUMM Compositions of the present invention can be administered to any animal having a ***Fc*** ***epsilon*** ***receptor*** or an IgE that binds to a therapeutic compound of the present invention or to a protein expressed by a. . .

SUMM . . . a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, ***human*** serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

SUMM . . . the blood of an animal at a constant rate sufficient to attain therapeutic dose levels of the composition to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in the animal. As used herein, a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response refers to cellular responses that occur when ***Fc*** ***epsilon*** ***receptor*** is complexed with IgE. For example, a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response includes release of biological mediators, such as histamine, prostaglandins and/or proteases, that can trigger clinical symptoms of allergy.. . .

SUMM . . . of skill in the art in accordance with the given condition of a patient. For example, to regulate an antigen-specific ***Fc*** ***epsilon*** ***receptor*** -mediated response, a therapeutic composition may be administered more frequently when an antigen is present in a patient's environment in high. . .

SUMM . . . can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into an equine ***Fc*** .sub..di-elect cons. R.alpha. protein or an equine ***Fc*** .sub..di-elect cons. R.alpha. RNA (e.g., antisense RNA, ribozyme, triple helix forms or RNA drug) in the animal. Nucleic acid molecules can. . .

SUMM . . . the recipient animal and directs the production of a protein or RNA nucleic acid molecule that is capable of reducing ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in the animal. For example, a recombinant virus comprising an equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecule of the present invention is administered according to a protocol that results in the animal producing an amount of protein or RNA sufficient to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. A preferred single dose of a recombinant virus of the present invention is from about 1.times.10.sup.4 to about. . .

SUMM . . . a therapeutic composition of the present invention includes recombinant cells of the present invention that comprises at least one equine ***Fc*** .sub..di-elect cons. R.alpha. of the present invention. Preferred recombinant cells for this embodiment include Salmonella, E. coli, Listeria, Mycobacterium, S. frugiperda,. . .

SUMM . . . an animal an effective amount of a therapeutic composition selected from the group consisting of an inhibitor of an equine ***Fc*** .sub..di-elect cons. R.alpha. and an equine ***Fc*** .sub..di-elect cons. R.alpha. protein (including homologs), wherein said equine ***Fc*** .sub..di-elect cons. R.alpha. is capable of binding to IgE. Suitable therapeutic compositions and methods of administration methods are disclosed herein. According. . . invention, a therapeutic composition and method of the present invention can be used to prevent or alleviate symptoms associated with ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

SUMM The efficacy of a therapeutic composition of the present invention to effect ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses can be tested using standard methods for detecting ***Fc*** receptor-mediated immunity including, but not limited to, immediate hypersensitivity, delayed hypersensitivity, antibody-dependent cellular cytotoxicity (ADCC), immune complex activity, mitogenic activity,. . .

SUMM An inhibitor of equine ***Fc*** .sub..di-elect cons. R.alpha. activity can be identified using equine ***Fc*** .sub..di-elect cons. R.alpha. proteins of the present invention by determining the ability of an inhibitor to prevent or disrupt complex formation between an equine ***Fc*** .sub..di-elect cons. R.alpha. protein and IgE. One embodiment of the present invention is a method to identify a compound capable of inhibiting equine ***Fc*** .sub..di-elect cons. R.alpha. activity. Such a method includes the steps of (a) contacting (e.g., combining, mixing) an isolated equine ***Fc*** .sub..di-elect cons. R.alpha. protein with a putative inhibitory compound under conditions in which,

in the absence of the compound, the equine ***Fc*** .sub..di-elect cons. R.alpha. protein has IgE binding activity, and (b) determining if the putative inhibitory compound inhibits the IgE binding activity.. . .

SUMM The present invention also includes a test kit to identify a compound capable of inhibiting equine ***Fc*** .sub..di-elect cons. R.alpha. activity. Such a test kit includes: an isolated equine ***Fc*** .sub..di-elect cons. R.alpha. protein having IgE binding activity or a complex of equine ***Fc*** .sub..di-elect cons. R.alpha. protein and IgE; and a means for determining the extent of inhibition of IgE binding activity in the. . .

DETD This example describes the isolation, by DNA hybridization, of a nucleic acid molecule encoding a ***Fc*** .sub..di-elect cons. R.alpha. chain from Equus caballus.

DETD . . . isolated from a horse buffy coat eDNA library by its ability to hybridize with a .sup.32 P-labeled cDNA encoding the ***human*** ***Fc*** .sub..di-elect cons. R.alpha. chain (Kochan et al., Nucleic Acids Res. 16:3584, 1988). The horse buffy coat cDNA library was prepared as. . .

DETD The horse buffy coat cDNA library was screened, using duplicate plaque filter lifts, with a .sup.32 P-labeled cDNA encoding the ***human*** ***Fc*** .sub..di-elect cons. R.alpha. chain under the following conditions. The filters were pre-hybridized and hybridized in a hybridization solution including 5.times. SSC,. . .

DETD This example describes the sequencing of an equine ***Fc*** .sub..di-elect cons. R.alpha. chain nucleic acid molecule of the present invention.

DETD . . . includes GenBank+EMBL+DDBJ+PDB. The highest scoring match of the homology search at the amino acid level was SwissProt accession number P12319: ***human*** high affinity IgE receptor .alpha.-chain, which was about 61% identical with SEQ ID NO:2. At the nucleotide level, the search was performed using SEQ ID NO:1, which was most similar to GenBank accession number X06948, ***human*** mRNA for immunoglobulin E receptor alpha chain, which was about 75% identical to SEQ ID NO:1.

DETD This Example demonstrates the production of an equine ***Fc*** .sub..di-elect cons. R.alpha. chain protein in eukaryotic cells.

DETD . . . of SEQ ID NO:1, operatively linked to baculovirus polyhedron transcription control sequences, was produced in the following manncr. An equine ***Fc*** .sub..di-elect cons. R.alpha. nucleic acid molecule-containing fragment of about 603 nucleotides was PCR amplified from neqFc.sub..di-elect cons. R.alpha..sub.1015 using sense primer. . .

DETD . . . as pFB-neqFc.sub..di-elect cons. R.alpha..sub.603. Translation of SEQ ID NO:11 indicates that the nucleic acid molecule neqFc.sub..di-elect cons. R.alpha..sub.603 encodes a ***Fc*** .sub..di-elect cons. R.alpha. protein of about 201 amino acids, referred to herein as PequFc.sub..di-elect cons. R.alpha..sub.201, having amino acid sequence SEQ. . .

DETD . . . cons. R.alpha..sub.603 can be cultured using conditions known to those skilled in the art in order to produce the equine ***Fc*** .sub..di-elect cons. R.alpha. protein, PequFc.sub..di-elect cons. R.alpha..sub.201 or a secreted form thereof.

CLM What is claimed is:
2. The nucleic acid molecule of claim 1, wherein said equine nucleio acid molecule encodes an equine ***Fc*** .sub..di-elect cons. R.alpha. protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:7. . .
12. A method to produce an equine ***Fc*** .sub..di-elect cons. R.alpha. protein, said method comprising culturing a cell transformed with a nucleic acid molecule encoding an equine ***Fc*** .sub..di-elect cons. R.alpha. protein, wherein said equine nucleic acid molecule hybridizes in a solution comprising 5.times. SSC, 5.times. Denhardts, 0.5% SDS,. . .

L13 ANSWER 23 OF 24 USPATFULL on STN
AN 1999:117451 USPATFULL
TI Feline ***Fc*** ***epsilon*** ***receptor*** alpha chain proteins and therapeutic uses thereof
IN Frank, Glenn R., Wellington, CO, United States
Porter, James P., Fort Collins, CO, United States
Rushlow, Keith E., Fort Collins, CO, United States

Wassom, Donald L., Fort Collins, CO, United States
Weber, Eric R., Fort Collins, CO, United States
PA Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI US 5958880 19990928
AI US 1996-768964 19961219 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Kemmerer, Elizabeth
LREP Heska Corporation
CLMN Number of Claims: 13
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2759
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes methods to detect IgE using such proteins and antibodies. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.
TI Feline ***Fc*** ***epsilon*** ***receptor*** alpha chain proteins and therapeutic uses thereof
AB The present invention relates to feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of. . . such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to mediate ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.
SUMM The present invention relates to feline ***Fc*** ***epsilon*** ***receptor*** alpha chain nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of. . .
SUMM Immunological stimulation can be mediated by IgE antibodies when IgE complexes with ***Fc*** ***epsilon*** ***receptors*** . ***Fc*** ***epsilon*** ***receptors*** are found on the surface of certain cell types, such as mast cells. Mast cells store biological mediators including histamine, prostaglandins and proteases. Release of these biological mediators is triggered when IgE antibodies complex with ***Fc*** ***epsilon*** ***receptors*** on the surface of a cell. Clinical symptoms result from the release of the biological mediators into the tissue of. . .
SUMM . . . cross-react with other antibody idiotypes, such as gamma isotype antibodies. Also, creation of reagents capable of inhibiting the activity of ***Fc*** ***epsilon*** ***receptors*** has been limited.
SUMM The discovery of the present invention includes a novel feline ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .epsilon.R.alpha.) protein and the use of such a protein to detect the presence of IgE in a putative IgE-containing composition; to identify inhibitors of biological responses mediated by a feline ***Fc*** .epsilon.R.alpha. protein; and as a therapeutic compound to prevent or treat clinical symptoms that result from feline ***Fc*** .epsilon.R.alpha.-mediated biological responses. When used in an assay to detect IgE, a feline ***Fc*** .epsilon.R.alpha. protein provides an advantage over, for example anti-IgE antibodies, to detect IgE because a feline ***Fc*** .epsilon.R.alpha. protein can bind to an IgE with more specificity (i.e., less idiotype cross-reactivity) and more sensitivity (i.e., affinity) than anti-IgE. . .
SUMM Prior investigators have disclosed the nucleic acid sequence for: the ***human*** ***Fc*** .epsilon.R alpha chain (Kochan et al., Nucleic Acids Res. 16:3584, 1988; Shimizu et al., Proc. Natl. Acad. Sci. USA 85:1907-1911, 1988; and Pang et al., J. Immunol. 151:6166-6174, 1993); the ***human*** ***Fc*** .epsilon.R beta chain (Kuster et al., J. Biol. Chem. 267:12782-12787, 1992); the ***human*** ***Fc*** .epsilon.R gamma chain (Kuster et al., J. Biol. Chem. 265:6448-6452, 1990); and the canine ***Fc*** .epsilon.R alpha chain (GenBank.TM.

accession number D16413). Although the subunits of ***human*** ***Fc*** .epsilon.R have been known as early as 1988, they have never been used to identify a feline ***Fc*** .epsilon.R. Similarly, even though the canine ***Fc*** .epsilon.R chain has been known since 1993, it has never been used to identify a feline ***Fc*** .epsilon.R. Moreover, the determination of ***human*** and canine ***Fc*** ***epsilon*** ***receptor*** sequences does not indicate, suggest or predict the cloning of a novel ***Fc*** .epsilon.R.alpha. gene from a different species, in particular, from a feline species.

SUMM . . . processes of the present invention are needed in the art that will provide specific detection of IgE and treatment of ***Fc*** ***epsilon*** ***receptor*** -mediated disease.

SUMM The present invention relates to a novel product and process for detecting IgE and protecting animals from ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. According to the present invention there are provided feline ***Fc*** .epsilon.R.alpha. proteins and mimetopes thereof; feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules, including those that encode such proteins; antibodies raised against such feline ***Fc*** .epsilon.R.alpha. proteins (i.e., anti-feline ***Fc*** .epsilon.R.alpha. antibodies); and other compounds that inhibit the ability of feline ***Fc*** .epsilon.R.alpha. protein to form a complex with IgE (i.e, inhibitory compounds or inhibitors).

SUMM . . . comprising such proteins, mimetopes, nucleic acid molecules, antibodies, and/or inhibitory compounds, as well as use of such therapeutic compositions to ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

SUMM One embodiment of the present invention is an isolated nucleic acid molecule encoding a feline ***Fc*** .epsilon.R.alpha. protein. The feline ***Fc*** .epsilon.R.alpha. protein preferably includes: proteins comprising amino acid sequences SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12 and SEQ ID. . . allelic variants of a nucleic acid molecules encoding a protein comprising any of the amino acid sequences. Particularly preferred feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules include: nucleic acid molecules comprising nucleic acid sequences SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4,. . .

SUMM The present invention also includes an isolated feline ***Fc*** .epsilon.R.alpha. protein. A preferrred feline ***Fc*** .epsilon.R.alpha. protein is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a nucleic acid sequence including SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:15 and SEQ ID NO:16. Particularly preferred feline ***Fc*** .epsilon.R.alpha. proteins include at least one of the following amino acid sequences: SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12. . .

SUMM The present invention also relates to recombinant molecules, recombinant viruses and recombinant cells that include feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules of the present invention. Also included are methods to produce such nucleic acid molecules, recombinant molecules, recombinant. . .

SUMM . . . detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated feline ***Fc*** .epsilon.R.alpha. molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .epsilon.R.alpha. molecule:IgE complex; and (b) determining the presence of IgE by detecting the ***Fc*** .epsilon.R.alpha. molecule:IgE complex, the presence of the ***Fc*** .epsilon.R.alpha. molecule:IgE complex indicating the presence of IgE. A preferred feline ***Fc*** .epsilon.R.alpha. molecule is one which a carbohydrate group of the feline ***Fc*** .epsilon.R.alpha. molecule is conjugated to biotin.

SUMM . . . IgE-containing composition under conditions suitable for formation of a recombinant cell:IgE complex, in which the recombinant cell comprises a feline ***Fc*** .epsilon.R.alpha. molecule; and (b) determining the presence of IgE by detecting the recombinant cell:IgE complex, the presence of the recombinant cell:IgE complex indicating the presence of IgE. A preferred method to detect IgE comprises: (a) immobilizing the ***Fc*** .epsilon.R.alpha. molecule on a substrate; (b) contacting the ***Fc*** .epsilon.R.alpha. molecule with the putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .epsilon.R.alpha. molecule:IgE complex bound to the substrate; (c) removing non-bound material from the substrate

under conditions that retain ***Fc*** .epsilon.R.alpha. molecule:IgE complex binding to the substrate; and (d) detecting the presence of the ***Fc*** .epsilon.R.alpha. molecule:IgE complex. Another preferred method to detect IgE comprises: (a) immobilizing a specific antigen on a substrate; (b) contacting the. . . binding to said substrate; and (d) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with said ***Fc*** .epsilon.R.alpha. molecule. Another preferred method to detect IgE comprises: (a) immobilizing an antibody that binds selectively to IgE on a substrate;. . . binding to the substrate; and (d) detecting the presence of the antibody:IgE complex by contacting the antibody:IgE complex with said ***Fc*** .epsilon.R.alpha. molecule. Another preferred method to detect IgE comprises: (a) immobilizing a putative IgE-containing composition on a substrate; (b) contacting the composition with the ***Fc*** .epsilon.R.alpha. molecule under conditions suitable for formation of a ***Fc*** .epsilon.R.alpha. molecule:IgE complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain ***Fc*** .epsilon.R.alpha. molecule:IgE complex binding to the substrate; and (d) detecting the presence of the ***Fc*** .epsilon.R.alpha. molecule:IgE complex.

SUMM . . . to the substrate; and (d) detecting the presence of the allergen:IgE complex by contacting said allergen:IgE complex with a feline ***Fc*** .epsilon.R.alpha. protein. Preferably, the flea allergen is a flea saliva antigen and more preferably flea saliva products and/or flea saliva proteins.

SUMM . . . a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a feline ***Fc*** .epsilon.R.alpha. protein and a means for detecting IgE. Another embodiment is a kit for detecting flea allergy dermatitis comprising a feline ***Fc*** .epsilon.R.alpha. protein and a flea allergen.

SUMM The present invention also includes an inhibitor that interferes with formation of a complex between feline ***Fc*** .epsilon.R.alpha. protein and IgE, in which the inhibitor is identified by its ability to interfere with the complex formation. A particularly preferred inhibitor includes a substrate analog of a feline ***Fc*** .epsilon.R.alpha. protein, a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein and a soluble portion of a feline ***Fc*** .epsilon.R.alpha. protein. Also included is a method to identify a compound that interferes with formation of a complex between feline ***Fc*** .epsilon.R.alpha. protein and IgE, the method comprising: (a) contacting an isolated feline ***Fc*** .epsilon.R.alpha. protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the feline ***Fc*** .epsilon.R.alpha. protein forms a complex with IgE; and (b) determining if the putative inhibitory compound inhibits the complex formation. A test kit is also includes to identify a compound capable of interfering with formation of a complex between a feline ***Fc*** .epsilon.R.alpha. protein and IgE, the test kit comprising an isolated feline ***Fc*** .epsilon.R.alpha. protein that can complex with IgE and a means for determining the extent of interference of the complex formation in. . .

SUMM Yet another embodiment of the present invention is a therapeutic composition that is capable of reducing ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. Such a therapeutic composition includes one or more of the following therapeutic compounds: an isolated feline ***Fc*** .epsilon.R.alpha. protein; a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a feline ***Fc*** .epsilon.R.alpha. gene; an isolated antibody that selectively binds to a feline ***Fc*** .epsilon.R.alpha. protein; and an inhibitor that interferes with formation of a complex between a feline ***Fc*** .epsilon.R.alpha. protein and IgE. A method of the present invention includes the step of administering to an animal a therapeutic composition. . .

SUMM Yet another embodiment of the present invention is a method to produce a feline ***Fc*** .epsilon.R.alpha. protein, the method comprising culturing a cell transformed with a nucleic acid molecule encoding a feline ***Fc*** .epsilon.R.alpha. protein.

SUMM The present invention provides for isolated feline ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .epsilon.R.alpha.) proteins, isolated feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules, antibodies directed against

feline ***Fc*** .epsilon.R.alpha. proteins and other inhibitors of feline ***Fc*** .epsilon.R.alpha. activity. As used herein, the terms isolated feline ***Fc*** .epsilon.R.alpha. proteins and isolated feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules refers to feline ***Fc*** .epsilon.R.alpha. proteins and feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules derived from cats and, as such, can be obtained from their natural source or can be produced. . . of the present invention are advantageous because they enable the detection of IgE and the inhibition of IgE or feline ***Fc*** .epsilon.R.alpha. protein activity associated with disease. As used herein, feline ***Fc*** epsilon alpha chain receptor protein can be referred to as ***Fc*** .epsilon.R.alpha. protein or ***Fc*** .epsilon.R.alpha. chain protein.

SUMM One embodiment of the present invention is an isolated protein comprising a feline ***Fc*** .epsilon.R.alpha. protein. It is to be noted that the term "a" or "an" entity refers to one or more of that. . .

SUMM As used herein, an isolated feline ***Fc*** .epsilon.R.alpha. protein can be a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. Preferably, a feline ***Fc*** .epsilon.R.alpha. protein comprises at least a portion of a feline ***Fc*** .epsilon.R.alpha. protein that binds to IgE, i.e., that is capable of forming a complex with an IgE.

SUMM A feline ***Fc*** .epsilon.R.alpha. protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to bind to IgE. Examples of feline ***Fc*** .epsilon.R.alpha. protein homologs include feline ***Fc*** .epsilon.R.alpha. proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted,. . .

SUMM Feline ***Fc*** .epsilon.R.alpha. protein homologs can be the result of natural allelic variation or natural mutation. Feline ***Fc*** .epsilon.R.alpha. protein homologs of the present invention can also be produced using techniques known in the art including, but not limited. . .

SUMM Isolated feline ***Fc*** .epsilon.R.alpha. proteins of the present invention have the further characteristic of being encoded by nucleic acid molecules that hybridize under stringent hybridization conditions to a gene encoding a feline ***Fc*** .epsilon.R.alpha. protein. As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules, including oligonucleotides, are. . .

SUMM As used herein, a feline ***Fc*** .epsilon.R.alpha. gene includes all nucleic acid sequences related to a natural feline ***Fc*** .epsilon.R.alpha. gene such as regulatory regions that control production of the feline ***Fc*** .epsilon.R.alpha. protein encoded by that gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. In one embodiment, a feline ***Fc*** .epsilon.R.alpha. gene of the present invention includes nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID. . .

SUMM . . . other nucleic acid and protein sequences presented herein) represent apparent nucleic acid sequences of certain nucleic acid molecules encoding feline ***Fc*** .epsilon.R.alpha. proteins of the present invention.

SUMM In another embodiment, a feline ***Fc*** .epsilon.R.alpha. gene can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:1, SEQ ID. . . NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:15 and/or SEQ ID NO:16. An allelic variant of a feline ***Fc*** .epsilon.R.alpha. gene is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including. . .

SUMM The minimal size of a ***Fc*** .epsilon.R.alpha. protein homolog of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of. . . length if they are AT-rich. As such, the minimal size of a nucleic acid molecule used to encode a feline ***Fc*** .epsilon.R.alpha. protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of a feline ***Fc*** .epsilon.R.alpha. protein homolog of the present invention is from about 4 to about 6 amino acids in length. There is no. . .

SUMM . . . of the cat family, including domestic cats, wild cats and zoo cats. Examples of cats from which to isolate feline ***Fc***

.epsilon.R.alpha. proteins of the present invention (including isolation of the natural protein or production of the protein by recombinant or synthetic. . . .

SUMM Suitable cat cells from which to isolate a feline ***Fc*** .epsilon.R.alpha. protein of the present invention include cells that have ***Fc*** .epsilon.R proteins. Preferred cat cells from which to obtain a feline ***Fc*** .epsilon.R.alpha. protein of the present invention include basophil cells, mast cells, mastocytoma cells, dendritic cells, B lymphocytes, macrophages, eosinophils, and/or monocytes. A feline ***Fc*** .epsilon.R.alpha. of the present invention is preferably obtained from mastocytoma cells, mast cells or basophil cells.

SUMM The present invention also includes mimetopes of feline ***Fc*** .epsilon.R.alpha. proteins of the present invention. As used herein, a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein of the present invention refers to any compound that is able to mimic the activity of such a feline ***Fc*** .epsilon.R.alpha. protein (e.g., ability to bind to IgE), often because the mimetope has a structure that mimics the feline ***Fc*** .epsilon.R.alpha.: protein. It is to be noted, however, that the mimetope need not have a structure similar to a feline ***Fc*** .epsilon.R.alpha. protein as long as the mimetope functionally mimics the protein. Mimetopes can be, but are not limited to: peptides that. . . nucleic acids; and/or any other peptidomimetic compounds. Mimetopes of the present invention can be designed using computer-generated structures of feline ***Fc*** .epsilon.R.alpha. proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides. . . other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., a feline IgE ***Fc*** domain or anti-feline ***Fc*** .epsilon.R.alpha. antibody). A mimetope can also be obtained by, for example, rational drug design. In a rational drug design procedure, the. . . for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of feline ***Fc*** .epsilon.R.alpha. mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex.TM. technology, peptides identified by random screening of peptide libraries and proteins identified. . . by phage display technology. A preferred mimetope is a peptidomimetic compound that is structurally and/or functionally similar to a feline ***Fc*** .epsilon.R.alpha. protein of the present invention, particularly to the IgE ***Fc*** domain binding site of the feline ***Fc*** .epsilon.R.alpha.protein. As used herein, the ***Fc*** domain of an antibody refers to the portion of an immunoglobulin that has ***Fc*** receptor binding effector function. Typically, the ***Fc*** domain of an IgE comprises the CH2 and CH3 domains of the heavy chain constant region.

SUMM According to the present invention, a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention refers to: a feline ***Fc*** .epsilon.R.alpha. protein, in particular a soluble feline ***Fc*** .epsilon.R.alpha. protein; a feline ***Fc*** .epsilon.R.alpha. homolog; a feline ***Fc*** .epsilon.R.alpha. mimetope; a feline ***Fc*** .epsilon.R.alpha. substrate analog; or a feline ***Fc*** .epsilon.R.alpha. peptide. Preferably, a feline ***Fc*** .epsilon.R.alpha. molecule binds to IgE.

SUMM One embodiment of a feline ***Fc*** .epsilon.R.alpha. protein of the present invention is a fusion protein that includes a feline ***Fc*** .epsilon.R.alpha. protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but. . . to, segments that can: enhance a protein's stability; act as an immunopotentiator to enhance an immune response against a feline ***Fc*** .epsilon.R.alpha. protein; and/or assist purification of a feline ***Fc*** .epsilon.R.alpha. protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired. . . protein, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the feline ***Fc*** .epsilon.R.alpha.-containing domain of the protein and can be susceptible to cleavage in order to enable straight-forward recovery of a feline ***Fc*** .epsilon.R.alpha. protein. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid molecule that encodes a protein including the fusion

segment attached to either the carboxyl and/or amino terminal end of a feline ***Fc*** .epsilon.R.alpha.-containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; ***Fc*** receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); a "tag" domain (e.g., at. . . the domain, such as monoclonal antibodies); and/or a linker and enzyme domain (e.g., alkaline phosphatase domain connected to a feline ***Fc*** .epsilon.R.alpha. protein by a linker). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding. . .

SUMM A preferred feline ***Fc*** .epsilon.R.alpha. protein of the present invention is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with at least one of the following nucleic acid molecules: nfelFc.sub..epsilon. R.alpha..sub.1069, nfelFc.sub..epsilon. R.alpha..sub.789,nfelFc.sub..epsilon. R.alpha..sub.714, nfelFc.sub..epsilon. R.alpha..sub.597,nfelFc.sub..epsi lon. R.alpha..sub.522. Preferably, the feline ***Fc*** .epsilon.R.alpha. protein binds to IgE. A further preferred isolated protein is encoded by a nucleic acid molecule that hybridizes under stringent. . .

SUMM . . . acid sequences reported in GenBank.TM. indicates that SEQ ID NO:2 showed the most homology, i.e., about 54% identity, with a ***Fc*** ***epsilon*** ***receptor*** alpha chain protein of Homo Sapiens (GenBank accession number J03605).

SUMM More preferred feline ***Fc*** .epsilon.R.alpha. proteins of the present invention include proteins comprising amino acid sequences that are at least about 60%, preferably at least. . .

SUMM More preferred feline ***Fc*** .epsilon.R.alpha. proteins of the present invention include proteins encoded by a nucleic acid molecule comprising at least a portion of nfelFc.sub..epsilon.. . . allelic variants of such nucleic acid molecules, the portion being capable of binding to IgE. More preferred is a feline ***Fc*** .epsilon.R.alpha. protein encoded by nfelFc.sub..epsilon. R.alpha..sub.1069, nfelFc.sub..epsilon. R.alpha..sub.789,nfelFc.sub..epsilon. R.alpha..sub.714, nfelFc.sub..epsilon. R.alpha..sub.597 and nfelFc.sub..epsilon. R.alpha..sub.522, or by an allelic variant of such nucleic acid molecules. Particularly preferred feline ***Fc*** .epsilon.R.alpha. proteins are PfelFc.sub..epsilon. R.alpha..sub.238, PfelFc.sub..epsilon. R.alpha..sub.263, PfelFc.sub..epsilon. R.alpha..sub.199 and PfelFc.sub..epsilon. R.alpha..sub.174.

SUMM In one embodiment, a preferred feline ***Fc*** .epsilon.R.alpha. A protein of the present invention is encoded by at least a portion of SEQ ID NO:1, SEQ ID NO:4,. . .

SUMM Also preferred is a feline ***Fc*** .epsilon.R.alpha. A protein encoded by an allelic variant of a nucleic acid molecule comprising at least a portion of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:11 and/or SEQ ID NO:14. Particularly preferred feline ***Fc*** .epsilon.R.alpha. proteins of the present invention include SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:12 and SEQ ID NO:13 (including,. . .

SUMM . . . embodiment of the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a feline ***Fc*** .epsilon.R.alpha. gene. The identifying characteristics of such a gene are heretofore described. A nucleic acid molecule of the present invention can include an isolated natural feline ***Fc*** .epsilon.R.alpha. gene or a homolog thereof, the latter of which is described in more detail below. A nucleic acid molecule of. . . nucleic acid molecule of the present invention is the minimal size that can form a stable hybrid with a feline ***Fc*** .epsilon.R.alpha. gene under stringent hybridization conditions.

SUMM . . . molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to ***human*** manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule of the present invention can be isolated from its natural source or can be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules can include, for example,

natural allelic variants and nucleic acid molecules modified by nucleotide insertions, deletions, substitutions,. . . a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode a feline ***Fc*** .epsilon.R.alpha. protein of the present invention or to form stable hybrids under stringent conditions with natural gene isolates.

SUMM A feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule homolog can be produced using a number of methods known to those skilled in the art (see,. . . mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologs can be selected by hybridization with a feline ***Fc*** .epsilon.R.alpha. gene or by screening for function of a protein encoded by the nucleic acid molecule (e.g., ability of a feline ***Fc*** .epsilon.R.alpha. protein to bind IgE).

SUMM . . . isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one feline ***Fc*** .epsilon.R.alpha. protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers. . . interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a feline ***Fc*** .epsilon.R.alpha. protein.

SUMM One embodiment of the present invention is a feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule that hybridizes under stringent hybridization conditions with nucleic acid molecule nfelFc.sub..epsilon. R.alpha..sub.1069 and preferably with a nucleic. . .

SUMM . . . acid sequences reported in GenBank indicates that SEQ ID NO:1 showed the most homology, i.e., about 77% identity a canine ***Fc*** ***epsilon*** ***receptor*** alpha chain gene.

SUMM Preferred feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules include nucleic acid molecules having a nucleic acid sequence that is at least about 80%, preferably at. . .

SUMM . . . ID NO:11, SEQ ID NO:14, SEQ ID NO:15 and/or SEQ ID NO:16, that is capable of hybridizing to a feline ***Fc*** .epsilon.R.alpha. gene of the present invention, as well as allelic variants thereof. A more preferred nucleic acid molecule includes the nucleic. . .

SUMM Knowing the nucleic acid sequences of certain feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of. . . (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules from other cats. Such nucleic acid molecules can be obtained in a variety of ways including screening. . .

SUMM . . . conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising feline ***Fc*** .epsilon.R.alpha. genes or other feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules. Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of. . . for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules or therapeutic reagents to inhibit feline ***Fc*** .epsilon.R.alpha. protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also includes the. . .

SUMM . . . is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulation of feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules of the present invention.

SUMM . . . the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed feline ***Fc*** .epsilon.R.alpha. protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences. . .

SUMM . . . that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include feline ***Fc*** .epsilon.R.alpha. nucleic acid molecules disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include nfelFc.sub..epsilon. R.alpha..sub.1069, nfelFc.sub..epsilon.. . .

SUMM . . . production of multivalent vaccines). Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing feline ***Fc*** .epsilon.R.alpha. proteins of the present invention or can be capable of producing such proteins after being

transformed with at least one. . . . cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., ***human*** , murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK.sup.31 cells and/or. . .

SUMM Isolated feline ***Fc*** .epsilon.R.alpha. proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins,. . . permit protein production. An effective medium refers to any medium in which a cell is cultured to produce a feline ***Fc*** .epsilon.R.alpha. protein of the present invention. Such a medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources,. . .

SUMM The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to a feline ***Fc*** .epsilon.R.alpha. protein of the present invention or a mimetope thereof (i.e., anti- feline ***Fc*** .epsilon.R.alpha. antibodies). As used herein, the term "selectively binds to" a feline ***Fc*** .epsilon.R.alpha. protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof. . . . the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc.; see, for example, Sambrook et al., ibid. An anti- feline ***Fc*** .epsilon.R.alpha. antibody preferably selectively binds to a feline ***Fc*** .epsilon.R.alpha. protein in such a way as to reduce the activity of that protein.

SUMM . . . antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce feline ***Fc*** .epsilon.R.alpha. proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not. . .

SUMM . . . are within the scope of the present invention. For example, such antibodies can be used (a) as tools to detect ***Fc*** ***epsilon*** ***receptor*** in the presence or absence of IgE and/or (b) as tools to screen expression libraries and/or to recover desired proteins. . . . proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target cytotoxic agents to cells having ***Fc*** ***epsilon*** ***receptors*** such as those disclosed herein in order to directly kill such cells. Targeting can be accomplished by conjugating (i.e., stably. . . in the art. Suitable cytotoxic agents are known to those skilled in the art. Antibodies of the present invention, including ***Fc*** .epsilon.R.alpha.-binding portions thereof, can also be used, for example, to inhibit binding of IgE to ***Fc*** ***epsilon*** ***receptors*** , to produce anti-feline ***Fc*** .epsilon.R.alpha. idiotypic antibodies, to purify cells having feline ***Fc*** .epsilon.R.alpha. proteins, to stimulate intracellular signal transduction through a feline ***Fc*** .epsilon.R.alpha.. and to identify cells having feline ***Fc*** .epsilon.R.alpha. proteins.

SUMM A feline ***Fc*** .epsilon.R.alpha. molecule of the present invention can include chimeric molecules comprising a portion of a feline ***Fc*** .epsilon.R.alpha. molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the ***Fc*** .epsilon.R.alpha. molecule portion binds to IgE in essentially the same manner as a ***Fc*** .epsilon.R.alpha. molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of an. . .

SUMM A feline ***Fc*** .epsilon.R.alpha. molecule of the present invention can be contained in a formulation, herein referred to as a ***Fc*** .epsilon.R.alpha. molecule formulation. For example, a feline ***Fc*** .epsilon.R.alpha. molecule can be combined with a buffer in which the feline ***Fc*** .epsilon.R.alpha. molecule is solubilized, and/or with a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a feline ***Fc*** .epsilon.R.alpha. molecule can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate. . . . not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be mixed with feline ***Fc*** .epsilon.R.alpha. molecules or conjugated (i.e., attached) to feline ***Fc*** .epsilon.R.alpha. molecules in such a manner as to not substantially interfere with the ability of the feline ***Fc*** .epsilon.R.alpha.

molecules to selectively bind to IgE.

SUMM A feline ***Fc*** .epsilon.R.alpha. protein of the present invention can be bound to the surface of a cell comprising the feline ***Fc*** .epsilon.R.alpha. protein. A preferred feline ***Fc*** .epsilon.R.alpha. protein-bearing cell includes a recombinant cell comprising a nucleic acid molecule encoding a feline ***Fc*** .epsilon.R.alpha. protein of the present invention. A more preferred recombinant cell of the present invention comprises a nucleic acid molecule that. . .

SUMM In addition, a feline ***Fc*** .epsilon.R.alpha. molecule formulation of the present invention can include not only a feline ***Fc*** .epsilon.R.alpha. molecule but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers. . .

SUMM . . . of the present invention is a method to detect IgE which includes the steps of: (a) contacting an isolated feline ***Fc*** .epsilon.R.alpha. molecule with a putative IgE-containing composition under conditions suitable for formation of a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex; and (b) detecting the presence of IgE by detecting the feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex. Presence of such a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex indicates that the animal is producing IgE. Preferred IgE to detect using a feline ***Fc*** .epsilon.R.alpha. molecule include feline IgE, canine IgE, equine IgE and ***human*** IgE, with feline IgE being particularly preferred. The present method can further include the step of determining whether an IgE complexed with a feline ***Fc*** .epsilon.R.alpha. protein is heat labile. Preferably, a heat labile IgE is determined by incubating an IgE at about 56.degree. C. for. . . or heartworm allergens. Moreover, the inventors believe that identification of heat labile IgE and non-heat labile IgE using a feline ***Fc*** .epsilon.R.alpha. protein suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE antibodies. As such, a feline ***Fc*** .epsilon.R.alpha. protein of the present invention may be useful for detecting molecules bound by the feline ***Fc*** .epsilon.R.alpha. protein but not identical to a known IgE.

SUMM . . . used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with a feline ***Fc*** .epsilon.R.alpha. molecule. Formation of a complex between a feline ***Fc*** .epsilon.R.alpha. molecule and an IgE refers to the ability of the feline ***Fc*** .epsilon.R.alpha. molecule to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between a feline ***Fc*** .epsilon.R.alpha. molecule and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures,. . .

SUMM . . . are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between feline ***Fc*** .epsilon.R.alpha. molecule and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in. . .

SUMM . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the feline ***Fc*** .epsilon.R.alpha. molecule or to a reagent that selectively binds to the feline ***Fc*** .epsilon.R.alpha. molecule or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable. . . compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin available from Pierce, Rockford, Ill.). According to the present invention, a ***detectable*** ***marker*** can be connected to a feline ***Fc*** .epsilon.R.alpha. molecule using, for example, chemical conjugation or recombinant DNA technology (e.g., connection of a fusion segment such as that described. . . binding domain; an immunoglobulin binding; a sugar binding domain; and a "tag"

domain). Preferably a carbohydrate group of the feline ***Fc*** .epsilon.R.alpha. molecule is chemically conjugated to biotin.

SUMM In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a feline ***Fc*** .epsilon.R.alpha. molecule that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a feline ***Fc*** .epsilon.R.alpha. molecule includes, but is not limited to, a radioactive label, a fluorescent label, an enzyme label, a chemiluminescent label, a chromophoric label or a ligand. A ***detectable*** ***marker*** is conjugated to a feline ***Fc*** .epsilon.R.alpha. molecule in such a manner as not to block the ability of the feline ***Fc*** .epsilon.R.alpha. molecule to bind to the IgE being detected. Preferably, a carbohydrate group of a feline ***Fc*** .epsilon.R.alpha. molecule is conjugated to biotin.

SUMM In another embodiment, a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex is detected by contacting a putative IgE-containing composition with a feline ***Fc*** .epsilon.R.alpha. molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the feline ***Fc*** .epsilon.R.alpha. molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, an antigen, an antibody and a lectin, depending upon which portion of the feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex is being detected. Preferred indicator molecules that are antibodies include, for example, anti-IgE antibodies and anti-feline ***Fc*** .epsilon.R.alpha. antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a feline ***Fc*** .epsilon.R.alpha. protein of the present invention produced in insect cells. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

SUMM In one preferred embodiment, a feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex is detected by contacting the complex with an indicator molecule that selectively binds to a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention. Examples of such indicator molecule includes, but are not limited to, an antibody that selectively binds to a feline ***Fc*** .epsilon.R.alpha. molecule (referred to herein as an anti-feline ***Fc*** .epsilon.R.alpha. antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to a feline ***Fc*** .epsilon.R.alpha. molecule. A feline ***Fc*** .epsilon.R.alpha. molecule conjugated to biotin is preferably detected using streptavidin, more preferably using ImmunoPure.RTM. NeutrAvidin (available from Pierce, Rockford, Ill.).

SUMM In another preferred embodiment, a feline ***Fc*** .epsilon.R.alpha. molecule:Ige complex is detected by contacting the complex with indicator molecule that selectively binds to an IgE antibody (referred to. . . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., a ***Fc*** receptor), and an antibody-binding complement protein. A preferred indicator molecule includes an anti-feline IgE antibody. As used herein, an anti-IgE. . . .

SUMM . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

SUMM A preferred immunoabsorbent assay method includes a step of either: (a) immobilizing a feline ***Fc*** .epsilon.R.alpha. molecule on a substrate prior to contacting a feline ***Fc*** .epsilon.R.alpha. molecule with a putative IgE-containing composition to form a feline ***Fc*** .epsilon.R.alpha. molecule-immobilized substrate; and (b) binding a putative IgE-containing composition on a substrate prior to contacting a feline ***Fc*** .epsilon.R.alpha. molecule with a putative IgE-containing composition to form a putative IgE-containing composition-bound substrate. Preferably, the substrate includes a non-coated substrate, a feline ***Fc*** .epsilon.R.alpha. molecule-immobilized substrate, an antigen-immobilized substrate or an anti-IgE antibody-immobilized substrate.

SUMM . . . whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention is used as a capture molecule when the feline ***Fc*** .epsilon.R.alpha. molecule is bound

on a substrate. Alternatively, a feline ***Fc*** .epsilon.R.alpha. molecule is used as an indicator molecule when the feline ***Fc*** .epsilon.R.alpha. molecule is not bound on a substrate. Suitable molecules for use as capture molecules or indicator molecules include, but are not limited to, a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

SUMM . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

SUMM . . . the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is a feline ***Fc*** .epsilon.R.alpha. molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

SUMM In one embodiment, a feline ***Fc*** .epsilon.R.alpha. molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or. . . biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the feline ***Fc*** .epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the feline ***Fc*** .epsilon.R.alpha. molecule:IgE complex. Preferably, the indicator molecule is conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a calorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . .

SUMM . . . if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. A feline ***Fc*** .epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the feline ***Fc*** .epsilon.R.alpha. molecule and the anti-IgE antibody:IgE complex. Preferably, the feline ***Fc*** .epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess feline ***Fc*** .epsilon.R.alpha. molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

SUMM . . . non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A feline ***Fc*** .epsilon.R.alpha. molecule is added to the substrate and incubated to allow formation of a complex between the feline ***Fc*** .epsilon.R.alpha. molecule and the IgE. Preferably, the feline ***Fc*** .epsilon.R.alpha. molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess feline ***Fc*** .epsilon.R.alpha. molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for. . .

SUMM . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a calorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . . which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case a feline ***Fc*** .epsilon.R.alpha. molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture reagent is. . .

SUMM . . . used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a feline ***Fc*** .epsilon.R.alpha. molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a capture. . .

SUMM . . . in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention and an isolated IgE known to bind to the feline ***Fc*** .epsilon.R.alpha. molecule. The absence of binding of the feline ***Fc*** .epsilon.R.alpha. molecule to the known IgE indicates the presence of IgE in the putative IgE-containing composition. The known IgE is preferably conjugated to a ***detectable*** ***marker*** .

SUMM . . . IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising a feline ***Fc*** .epsilon.R.alpha. protein and a means for detecting an IgE. Suitable and preferred feline ***Fc*** .epsilon.R.alpha. protein are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the feline ***Fc*** .epsilon.R.alpha. protein or to an IgE. A preferred kit of the present invention further comprises a detection means including one or. . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a feline ***Fc*** .epsilon.R.alpha. protein (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including canines, felines and/or equines.

SUMM . . . invention is a general allergen kit comprising an allergen common to all regions of the United States and a feline ***Fc*** .epsilon.R.alpha. protein of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that are. . .

SUMM . . . as cod, halibut or and tuna, egg, milk, Brewer's yeast, whole wheat, corn, soybean, cheese and rice, and a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

SUMM . . . present invention is a therapeutic composition that, when administered to an animal in an effective manner, is capable of reducing ***Fc*** receptor mediated reactions associated with diseases related to biological responses involving ***Fc*** receptor function. A therapeutic composition of the present invention can include: an isolated feline ***Fc*** .epsilon.R.alpha. protein, or homolog thereof; a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein; an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a feline ***Fc*** .epsilon.R.alpha. gene; an isolated antibody that selectively binds to a feline ***Fc*** .epsilon.R.alpha. protein; and/or an inhibitor that interferes with formation of a complex between a feline ***Fc*** .epsilon.R.alpha. protein and IgE.

SUMM One embodiment of a therapeutic composition of the present invention is a therapeutic compound comprising a feline ***Fc*** .epsilon.R.alpha. molecule of the present invention, that binds to an IgE. According to the present invention, a feline ***Fc*** .epsilon.R.alpha. molecule competes for IgE with naturally-occurring ***Fc*** ***epsilon*** ***receptors*** , particularly those on mastocytoma cells, mast cells or basophils, so that IgE is bound to the administered feline ***Fc*** .epsilon.R.alpha. molecule and thus is unable to bind to ***Fc*** ***epsilon*** ***receptor*** on a cell, thereby inhibiting mediation of a biological response. Preferred feline ***Fc*** .epsilon.R.alpha. molecule for use in a therapeutic composition comprises a feline ***Fc*** .epsilon.R.alpha. protein, or homolog thereof, as described herein, particularly a fragment thereof, which binds to IgE. Feline ***Fc*** .epsilon.R.alpha. molecules for use in a therapeutic composition can be in a monovalent and/or multivalent form, so long as the feline ***Fc*** .epsilon.R.alpha. molecule is capable of binding to IgE. A more preferred feline ***Fc*** .epsilon.R.alpha. molecule for use in a therapeutic composition includes a soluble fragment of a feline ***Fc*** .epsilon.R.alpha. protein. A preferred feline ***Fc*** .epsilon.R.alpha. protein is encoded by nfelFc.sub..epsilon. R.alpha..sub.522 and an even more preferred feline ***Fc*** .epsilon.R.alpha. protein is PfelFc.sub..epsilon. R.alpha..sub.174.

SUMM . . . therapeutic composition of the present invention comprises a therapeutic compound that interferes with the formation of a complex between feline ***Fc*** .epsilon.R.alpha. protein and IgE, usually by binding to or otherwise interacting with or otherwise modifying the

feline ***Fc*** .epsilon.R.alpha. A protein's IgE binding site. Feline ***Fc*** .epsilon.R.alpha. protein inhibitors can also interact with other regions of the feline ***Fc*** .epsilon.R.alpha. protein to inhibit feline ***Fc*** .epsilon.R.alpha. protein activity, for example, by allosteric interaction. An inhibitor of a feline ***Fc*** .epsilon.R.alpha. protein can interfere with ***Fc*** .epsilon.R.alpha. protein and IgE complex formation by, for example, preventing formation of a ***Fc*** .epsilon.R.alpha. protein and IgE complex or disrupting an existing ***Fc*** .epsilon.R.alpha. protein and IgE complex causing the ***Fc*** .epsilon.R.alpha. protein and IgE to dissociate. An inhibitor of a feline ***Fc*** .epsilon.R.alpha. protein is usually a relatively small. Preferably, a feline ***Fc*** .epsilon.R.alpha. protein inhibitor of the present invention is identified by its ability to bind to, or otherwise interact with, a feline ***Fc*** .epsilon.R.alpha. protein, thereby interfering with the formation of a complex between a feline ***Fc*** .epsilon.R.alpha. protein and IgE.

SUMM Preferred inhibitors of a feline ***Fc*** .epsilon.R.alpha. protein of the present invention include, but are not limited to, a substrate analog of a feline ***Fc*** .epsilon.R.alpha. protein, a mimetope of a feline ***Fc*** .epsilon.R.alpha. protein, a soluble (i.e., secreted form of a feline ***Fc*** .epsilon.R.alpha. protein) portion of a feline ***Fc*** .epsilon.R.alpha. protein that binds to IgE, and other molecules that bind to a feline ***Fc*** .epsilon.R.alpha. protein (e.g., to an allosteric site) in such a manner that IgE-binding activity of the feline ***Fc*** .epsilon.R.alpha. protein is inhibited. A feline ***Fc*** .epsilon.R.alpha. protein substrate analog refers to a compound that interacts with (e.g., binds to, associates with, modifies) the IgE-binding site of a feline ***Fc*** .epsilon.R.alpha. protein. A preferred feline ***Fc*** .epsilon.R.alpha. protein substrate analog inhibits IgE-binding activity of a feline ***Fc*** .epsilon.R.alpha. protein. Feline ***Fc*** .epsilon.R.alpha. protein substrate analogs can be of any inorganic or organic composition, and, as such, can be, but are not limited to, peptides, nucleic acids, and peptidomimetic compounds. Feline ***Fc*** .epsilon.R.alpha. protein substrate analogs can be, but need not be, structurally similar to a feline ***Fc*** .epsilon.R.alpha. protein's natural substrate (e.g., IgE) as long as they can interact with the active site (e.g., IgE-binding site of that feline ***Fc*** .epsilon.R.alpha.). Feline ***Fc*** .epsilon.R.alpha. protein substrate analogs can be designed using computer-generated structures of feline ***Fc*** .epsilon.R.alpha. proteins of the present invention or computer structures of, for example, the ***Fc*** domain of IgE. Substrate analogs can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides, peptidomimetic. . . inorganic or organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner, (e.g., a feline ***Fc*** .epsilon.R.alpha. protein or anti-feline ***Fc*** .epsilon.R.alpha. idiotypic antibody). A preferred feline ***Fc*** .epsilon.R.alpha. protein substrate analog is a peptidomimetic compound (i.e., a compound that is structurally and/or functionally similar to a natural substrate of a feline ***Fc*** .epsilon.R.alpha. protein of the present invention, particularly to the region of the substrate that binds to a feline ***Fc*** .epsilon.R.alpha. protein, but that inhibits IgE binding upon interacting with the IgE binding site).

SUMM Feline ***Fc*** .epsilon.R.alpha. molecules, as well as other inhibitors and therapeutic compounds, can be used directly as compounds in compositions of the present. . .

SUMM In one embodiment, a therapeutic composition of the present invention can be used to reduce a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response in an animal by administering such a composition to an animal. Preferably, an animal is treated by administering. . . therapeutic composition of the present invention in such a manner that a therapeutic compound (e.g., an inhibitor of a feline ***Fc*** .epsilon.R.alpha. protein, an anti-feline ***Fc*** .epsilon.R.alpha. antibody, an inhibitor of IgE, or nucleic acid molecules encoding feline ***Fc*** .epsilon.R.alpha. proteins) binds to an IgE or a ***Fc*** ***epsilon*** ***receptor*** in the animal. Such administration could be by a variety of routes known to those skilled in the art including,. . .

SUMM Compositions of the present invention can be administered to any animal having a ***Fc*** ***epsilon*** ***receptor*** or an IgE that binds to a therapeutic compound of the present invention or to a protein expressed by a. . .

SUMM . . . a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, ***human*** serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

SUMM . . . the blood of an animal at a constant rate sufficient to attain therapeutic dose levels of the composition to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in the animal. As used herein, a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response refers to cellular responses that occur when ***Fc*** ***epsilon*** ***receptor*** is complexed with IgE. For example, a ***Fc*** ***epsilon*** ***receptor*** -mediated biological response includes release of biological mediators, such as histamine, prostaglandins and/or proteases, that can trigger clinical symptoms of allergy.. . .

SUMM . . . of skill in the art in accordance with the given condition of a patient. For example, to regulate an antigen-specific ***Fc*** ***epsilon*** ***receptor*** -mediated response, a therapeutic composition may be administered more frequently when an antigen is present in a patient's environment in high. . .

SUMM . . . can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a feline ***Fc*** .epsilon.R.alpha. protein or a feline ***Fc*** .epsilon.R.alpha. RNA (e.g., antisense RNA, ribozyme, triple helix forms or RNA drug) in the animal. Nucleic acid molecules can be delivered. . .

SUMM . . . the recipient animal and directs the production of a protein or RNA nucleic acid molecule that is capable of reducing ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in the animal. For example, a recombinant virus comprising a feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule of the present invention is administered according to a protocol that results in the animal producing an amount of protein or RNA sufficient to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses. A preferred single dose of a recombinant virus of the present invention is from about 1.times.10.sup.4 to about. . .

SUMM . . . a therapeutic composition of the present invention includes recombinant cells of the present invention that comprises at least one feline ***Fc*** .epsilon.R.alpha. of the present invention. Preferred recombinant cells for this embodiment include Salmonella, E. coli, Listeria, Mycobacterium, S. frugiperda, yeast, (including. . .

SUMM . . . an animal an effective amount of a therapeutic composition selected from the group consisting of an inhibitor of a feline ***Fc*** .epsilon.R.alpha. and a feline ***Fc*** .epsilon.R.alpha. protein (including homologs), wherein said feline ***Fc*** .epsilon.R.alpha. is capable of binding to IgE. Suitable therapeutic compositions and methods of administration methods are disclosed herein. According to the. . . invention, a therapeutic composition and method of the present invention can be used to prevent or alleviate symptoms associated with ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses.

SUMM The efficacy of a therapeutic composition of the present invention to effect ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses can be tested using standard methods for detecting ***Fc*** receptor-mediated immunity including, but not limited to, immediate hypersensitivity, delayed hypersensitivity, antibody-dependent cellular cytotoxicity (ADCC), immune complex activity, mitogenic activity,. . .

SUMM An inhibitor of feline ***Fc*** .epsilon.R.alpha. activity can be identified using feline ***Fc*** .epsilon.R.alpha. proteins of the present invention by determining the ability of an inhibitor to prevent or disrupt complex formation between a feline ***Fc*** .epsilon.R.alpha. protein and IgE. One embodiment of the present invention is a method to identify a compound capable of inhibiting feline ***Fc*** .epsilon.R.alpha.. activity. Such a method includes the steps of (a) contacting (e.g., combining, mixing) an isolated feline ***Fc*** .epsilon.R.alpha. protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the feline ***Fc*** .epsilon.R.alpha. protein has IgE binding activity, and (b)

determining if the putative inhibitory compound inhibits the IgE binding activity. Putative inhibitory. . .

SUMM The present invention also includes a test kit to identify a compound capable of inhibiting feline ***Fc*** .epsilon.R.alpha. activity. Such a test kit includes: an isolated feline ***Fc*** .epsilon.R.alpha. protein having IgE binding activity or a complex of feline ***Fc*** .epsilon.R.alpha. protein and IgE; and a means for determining the extent of inhibition of IgE binding activity in the presence of. . .

DETD This example describes the isolation, by DNA hybridization, of a nucleic acid molecule encoding a feline ***Fc*** ***epsilon*** ***receptor*** alpha chain (***Fc*** .epsilon.R.alpha.) protein from Felis domesticus.

DETD A feline ***Fc*** .epsilon.R.alpha. nucleic acid molecule was isolated from a feline (Felis domesticus) mastocytoma cDNA library by hybridizing the library with a mixture of .sup.32 P-labeled cDNA molecules encoding ***human*** and canine ***Fc*** ***epsilon*** ***receptor*** alpha chains, respectively. A feline mastocytoma cDNA library was prepared as follows. Total RNA was extracted from approximately 1.5 grams. . . the feline mastocytoma cDNA library was screened, using duplicate plaque lifts, with a mixture of .sup.32 P-labeled cDNAs encoding the ***human*** ***Fc*** ***epsilon*** ***receptor*** alpha chain (Kochan et al., Nucleic Acids Res., 16:3584, 1988) and the canine ***Fc*** ***epsilon*** ***receptor*** alpha chain (Hayashi et al., GenBank accession number D16413, 1993), repsectively. A plaque purified clone identified using the above screening. . .

DETD This Example demonstrates the production of secreted feline ***Fc*** .epsilon.R.alpha. chain protein in eukaryotic cells.

DETD To produce a secreted form of the extracellular domain of the feline ***Fc*** .epsilon.R.alpha. chain, the hydrophobic transmembrane domain and the cytoplasmic tail of the feline ***Fc*** .epsilon.R.alpha. chain encoded by nfelFc.sub..epsilon. R.alpha..sub.1069 were removed as follows. A feline ***Fc*** .epsilon.R.alpha. chain extracellular domain nucleic acid molecule-containing a fragment of about 597 nucleotides was PCR amplified from nfelFc.sub..epsilon. R.alpha..sub.1069 using a. . . pVL-nfelFc.sub..epsilon. R.alpha..sub.597. Nucleic acid molecule Bv-nfelFc.sub..epsilon. R.alpha..sub.597 contained an about 597 nucleotide fragment encoding the extracellular domain of the feline ***Fc*** .epsilon.R.alpha. chain, extending from about nucleotide 65 through about 661 of SEQ ID NO:1, denoted herein as nucleic acid molecule nfelFc.sub..epsilon.. . . acid sequence denoted SEQ ID NO:11. Translation of SEQ ID NO:11 indicates that nucleic acid molecule nfelFc.sub..epsilon. R.alpha..sub.597 encodes a ***Fc*** .epsilon.R.alpha. protein of about 199 amino acids, referred to herein as PfelFc.sub..epsilon. R.alpha..sub.199, having amino acid sequence SEQ ID NO:12. Nucleic acid molecule nfelFc.sub..epsilon. R.alpha..sub.597 encodes a secretable form of the feline ***Fc*** .epsilon.R.alpha. chain. The processed protein product encoded by nfelFc.sub..epsilon. R.alpha..sub.597 is about 174 amino acids and does not possess a leader. . . .

DETD . . . frugiperda:pVL-nfelFc.sub..epsilon. R.alpha..sub.597. S. frugiperda:pVL-nfelFc.sub..epsilon. R.alpha..sub.597 is cultured using techniques known to those skilled in the art to produce a feline ***Fc*** .epsilon.R.alpha. protein PfelFc.sub..epsilon. R.alpha..sub.199.

CLM What is claimed is:

1. An isolated feline ***Fc*** .sub..epsilon. R.alpha. protein, wherein said isolated protein is isolated from a cell with which it naturally occurs, and wherein said protein. . . function selected from the group consisting of the ability to bind to an antibody that binds to a naturally-occurring feline ***Fc*** .sub..epsilon. R.alpha. protein and the ability to bind to IgE; (b) a protein comprising at least one epitope of the soluble. . .

4. The protein of claim 1, wherein said feline ***Fc*** .epsilon.R.alpha. protein comprises a protein selected from the group consisting of PfelFc.sub..epsilon. R.alpha..sub.238, PfelFc.sub..epsilon. R.alpha..sub.263, PfelFc.sub..epsilon. R.alpha..sub.199 and PfelFc.sub..epsilon. R.alpha..sub.174.

5. A therapeutic composition that, when administered to an animal,

reduces ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses, said therapeutic composition comprising an isolated soluble portion of a feline ***Fc*** .sub..epsilon. R.alpha. protein that is soluble and binds to IgE and an excipient, wherein said protein is selected from the group. . . a function selected from the group consisting of the ability to bind to an antibody that binds to a feline ***Fc*** .sub..epsilon. R.alpha. protein and the ability to bind to IgE; (b) a protein comprising at least one epitope of the soluble. . .

6. The composition of claim 5, wherein said soluble portion of a feline ***Fc*** .sub..epsilon. R.alpha. protein comprises a peptide that is a portion of a feline ***Fc*** .sub..epsilon. R.alpha. protein that binds to IgE.

8. A method to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in an animal comprising administering to an animal an effective amount of a therapeutic composition comprising an isolated soluble portion of a feline ***Fc*** .sub..epsilon. R.alpha. protein that is soluble and binds to IgE and an excipient, wherein said protein is selected from the group. . . a function selected from the group consisting of the ability to bind to an antibody that binds to a feline ***Fc*** .sub..epsilon. R.alpha. protein and the ability to bind to IgE; (b) a protein comprising at least one epitope of the soluble. . .

9. The method of claim 8, wherein said soluble portion of a feline ***Fc*** .sub..epsilon. R.alpha. protein comprises a peptide of a feline ***Fc*** .sub..epsilon. R.alpha. protein that binds to IgE.

11. An isolated feline ***Fc*** .sub..epsilon. R.alpha. protein, wherein said isolated protein is isolated from a cell with which it naturally occurs, and wherein said protein. . . function selected from the group consisting of the ability to bind to an antibody that binds to a naturally-occurring feline ***Fc*** .sub..epsilon. R.alpha. protein and the ability to bind to IgE; (b) a protein comprising at least one epitope of the soluble. . .

12. A therapeutic composition that, when administered to an animal reduces ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses, said therapeutic composition comprising an isolated soluble portion of a feline ***Fc*** .sub..epsilon. R.alpha. protein that is soluble and binds to IgE and an excipient, wherein said protein is selected from the group. . . function selected from the group consisting of the ability to bind to an antibody that binds to a naturally-occurring feline ***Fc*** .sub..epsilon. R.alpha. protein and the ability to bind to IgE; (b) a protein comprising at least one epitope of the soluble. . .

13. A method to reduce ***Fc*** ***epsilon*** ***receptor*** -mediated biological responses in an animal comprising administering to an animal an effective amount of a therapeutic composition comprising an isolated soluble portion of a feline ***Fc*** .sub..epsilon. R.alpha. protein that is soluble and binds to IgE and an excipient, wherein said protein is selected from the group. . . function selected from the group consisting of the ability to bind to an antibody that binds to a naturally-occurring feline ***Fc*** .sub..epsilon. R.alpha. protein and the ability to bind to IgE; (b) a protein comprising at least one epitope of the soluble. . .

L13 ANSWER 24 OF 24 USPATFULL on STN

AN	1999:102683 USPATFULL
TI	Method to detect IgE
IN	Frank, Glenn R., Wellington, CO, United States Porter, James P., Fort Collins, CO, United States Rushlow, Keith E., Fort Collins, CO, United States Wassom, Donald L., Fort Collins, CO, United States
PA	Heska Corporation, Fort Collins, CO, United States (U.S. corporation)
PI	US 5945294 19990831
AI	US 1996-756387 19961126 (8)
DT	Utility
FS	Granted
EXNAM	Primary Examiner: Housel, James; Assistant Examiner: Swartz, Rodney P.
LREP	Heska Corporation
CLMN	Number of Claims: 77

ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 2155
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention includes a method to detect IgE using a ***human*** ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon. R) to detect IgE antibodies in a biological sample from a cat, a dog, or a horse. The present invention also relates to kits to perform such methods.

AB The present invention includes a method to detect IgE using a ***human*** ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon. R) to detect IgE antibodies in a biological sample from a cat, a dog, or a horse. The present invention. . . .

SUMM Until the discovery of the present invention, detection of IgE in samples obtained from non- ***human*** animals has been hindered by the absence of suitable reagents for detection of IgE. Various compounds have been used to. . . . with other antibody idotypes, such as gamma isotype antibodies. The discovery of the present invention includes the use of a ***Fc*** ***epsilon*** ***receptor*** (***Fc*** .sub..epsilon. R) molecule to detect the presence of IgE in a putative IgE-containing composition. A ***Fc*** .sub..epsilon. R molecule provides an advantage over, for example anti-IgE antibodies, to detect IgE because a ***Fc*** .sub..epsilon. R molecule can bind to an IgE with more specificity (i.e., less isotype cross-reactivity) and more sensitivity (i.e., affinity) than. . . .

SUMM Lowenthal et al., 1993, Annals of Allergy 71:481-484, disclose that dog serum can transfer cutaneous reactivity to a ***human*** . While it is possible that Lowenthal et al. properly teach the binding of ***human*** ***Fc*** .sub..epsilon. R to canine IgE. Lowenthal et al., however, do not provide data defining the particular cellular proteins responsible for the. . . . by Lowenthal et al. is merely an interpretation. In addition, Lowenthal et al. do not teach the use of purified ***human*** ***Fc*** .sub..epsilon. R to detect canine IgE. The subunits of ***human*** ***Fc*** .sub..epsilon. R have been known as early as 1988 and have never been used to detect canine, feline or equine IgE. Indeed, U.S. Pat. No. 4,962,035, to Leder et al., issued Oct. 9, 1990, discloses ***human*** ***Fc*** .sub..epsilon. R but does not disclose the use of such a ***human*** ***Fc*** .sub..epsilon. R to detect ***human*** or non- ***human*** IgE. The use of purified ***human*** ***Fc*** .sub..epsilon. R avoids complications presented by use of ***Fc*** .sub..epsilon. R bound to a cell, such as non-specific binding of the ***Fc*** .sub..epsilon. R-bearing cell due to additional molecules present on the cell membrane. That purified ***human*** ***Fc*** .sub..epsilon. R detects non- ***human*** IgE is unexpected because inter-species binding between a ***Fc*** .sub..epsilon. R and an IgE is not predictable. For example, ***human*** ***Fc*** .sub..epsilon. R binds to rat IgE but rat ***Fc*** .sub..epsilon. R does not bind to ***human*** IgE.

SUMM The high affinity ***Fc*** .sub..epsilon. R consists of three protein chains, alpha, beta and gamma. Prior investigators have disclosed the nucleic acid sequence for: the. . . .

SUMM Thus, methods and kits are needed in the art that will provide specific detection of non- ***human*** IgE.

SUMM . . . that detect IgE. One embodiment of the present invention is a method to detect IgE comprising: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon. R molecule:IgE complex, wherein the IgE is selected from the group consisting of canine IgE, feline IgE and equine IgE; and (b) determining the presence of IgE by detecting the ***Fc*** .sub..epsilon. R molecule:IgE complex, the presence of the ***Fc*** .sub..epsilon. R molecule:IgE complex indicating the presence of IgE. A preferred ***Fc*** .sub..epsilon. R molecule in which a carbohydrate group of the ***Fc*** .sub..epsilon. R molecule is conjugated to biotin.

SUMM . . . conditions suitable for formation of a recombinant cell:IgE complex, in which the recombinant cell includes: a recombinant cell expressing a ***human*** ***Fc*** .sub..epsilon. R molecule; and a recombinant cell expressing an antibody that binds selectively to an IgE including canine IgE, feline IgE. . . .

SUMM . . . binding to the substrate; and (c) detecting the presence of the antigen:IgE complex by contacting the antigen:IgE complex with a ***Fc*** .sub..epsilon. R molecule. Preferably, the flea allergen is a flea saliva antigen and more preferably flea saliva products and/or flea saliva. . .

SUMM . . . includes a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and a means for detecting an IgE including canine IgE, feline IgE and equine IgE. Another embodiment is a general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule. Another embodiment is a kit for detecting flea allergy dermatitis comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and a flea allergen.

SUMM Another embodiment of the present invention is an isolated ***human*** ***Fc*** receptor (***Fc*** .sub..epsilon. R) alpha chain protein, in which a carbohydrate group of the ***Fc*** .sub..epsilon. R alpha chain protein is conjugated to biotin. A preferred ***Fc*** .sub..epsilon. R alpha chain protein comprises PhFc.sub..epsilon. R.alpha..sub.172 -BIOT.

DRWD FIG. 1 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect canine IgE antibodies.

DRWD FIG. 2 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect plant allergen-specific canine IgE antibodies.

DRWD FIG. 3 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect ***human*** or canine IgE antibodies.

DRWD FIG. 4 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect flea allergen-specific canine IgE antibodies.

DRWD FIG. 5 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect flea allergen-specific and heartworm antigen-specific canine IgE antibodies.

DRWD FIG. 6 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect flea saliva-specific canine IgE antibodies.

DRWD FIG. 7 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect heartworm antigen-specific feline IgE antibodies.

DRWD FIG. 8 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect heartworm antigen-specific feline IgE antibodies.

DRWD FIG. 9 depicts ELISA results using biotinylated alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect antigen-specific equine IgE antibodies.

DRWD FIG. 10 depicts ELISA results using basophilic leukemia cells expressing alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect canine IgE antibodies in sera from heartworm-infected dogs.

DRWD FIG. 11 depicts ELISA results using basophilic leukemia cells expressing alpha chain of ***human*** ***Fc*** .sub..epsilon. R to detect canine IgE antibodies in sera from flea saliva sensitized dogs.

DETD The present invention relates to the discovery that purified high affinity ***human*** ***Fc*** ***epsilon*** ***receptor*** (i.e., ***Fc*** .sub..epsilon. RI; referred to herein as ***Fc*** .sub..epsilon. R) can be used in certain non- ***human*** (i.e., canine, feline or equine) epsilon immunoglobulin (referred to herein as IgE or IgE antibody)-based detection (e.g., diagnostic, screening) methods and kits. The use of ***human*** ***Fc*** .sub..epsilon. R to detect non- ***human*** IgE is unexpected because canine, feline and equine immune systems are different from the ***human*** immune system, as well as from each other (i.e., molecules important to the immune system usually are species specific).

DETD One embodiment of the present invention is a method to detect a non- ***human*** IgE using an isolated ***human*** ***Fc*** .sub..epsilon. R molecule. It is to be noted that the term "a" entity or "an" entity refers to one or more. . .

DETD According to the present invention, an isolated, or biologically pure,

Fc .sub..epsilon. R molecule, is a molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the molecule has been purified. An isolated ***human*** ***Fc*** .sub..epsilon. R molecule of the present invention can be obtained from its natural source (e.g., from a ***human*** mast cell), can be produced using recombinant DNA technology or can be produced by chemical synthesis.

DETD A ***Fc*** .sub..epsilon. R molecule (also referred to herein as ***Fc*** .sub..epsilon. R or ***Fc*** .sub..epsilon. R protein) of the present invention can be a full-length protein, a portion of a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. A ***Fc*** .sub..epsilon. R molecule of the present invention can comprise a complete ***Fc*** .sub..epsilon. R (i.e., alpha, beta and gamma ***Fc*** .sub..epsilon. R chains), an alpha ***Fc*** .sub..epsilon. R chain (also referred to herein as ***Fc*** .sub..epsilon. R a chain) or portions thereof. Preferably, a ***Fc*** .sub..epsilon. R molecule comprises at least a portion of a ***Fc*** .sub..epsilon. R a chain that binds to IgE, i.e., that is capable of forming an immunocomplex with an IgE constant region. Preferably, a ***Fc*** .sub..epsilon. R molecule of the present invention binds to IgE with an affinity of about K.sub.A .apprxeq.10.sup.8, more preferably with an. . .

DETD An isolated ***Fc*** .sub..epsilon. R molecule of the present invention, including a homolog, can be identified in a straight-forward manner by the ***Fc*** .sub..epsilon. R molecule's ability to form an immunocomplex with an IgE. Examples of ***Fc*** .sub..epsilon. R homologs include ***Fc*** .sub..epsilon. R proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide),. . .

DETD ***Fc*** .sub..epsilon. R homologs can be the result of natural allelic variation or natural mutation. ***Fc*** .sub..epsilon. R homologs of the present invention can also be produced using techniques known in the art including, but not limited. . .

DETD According to the present invention, a ***human*** ***Fc*** .sub..epsilon. R .alpha. chain of the present invention is encoded by at least a portion of the nucleic acid sequence of the coding strand of a cDNA encoding a full-length ***Fc*** .sub..epsilon. R .alpha. chain protein represented herein as SEQ ID NO: 1, the portion at least encoding the IgE binding site of the ***Fc*** .sub..epsilon. R .alpha. chain protein. The double-stranded nucleic acid molecule including both the coding strand having SEQ ID NO: 1 and. . . determined by one skilled in the art and is shown herein as SEQ ID NO:3) is referred to herein as ***Fc*** .sub..epsilon. R nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.1198. Translation of SEQ ID NO: 1 suggests that nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.1198 encodes a full-length ***Fc*** .sub..epsilon. R .alpha. chain protein of about 257 amino acids, referred to herein as PhFc.sub..epsilon. R.alpha..sub.257, represented by SEQ ID NO:2,. . . skilled in the art to make modifications to the respective nucleic acid molecules and proteins to, for example, develop a ***Fc*** .sub..epsilon. R .alpha. chain protein with increased solubility and/or a truncated protein (e.g., a peptide) capable of detecting IgE, e.g., PhFc.sub..epsilon. R.alpha..sub.197 and PhFc.sub..epsilon. R.alpha..sub.172. Preferred ***Fc*** .sub..epsilon. R molecules include PhFc.sub..epsilon. R.alpha..sub.257, PhFc.sub..epsilon. R.alpha..sub.197, PhFc.sub..epsilon. R.alpha..sub.232 and PhFc.sub..epsilon. R.alpha..sub.172. Preferred nucleic acid molecules to encode a ***Fc*** .sub..epsilon. R molecules include nhFc.sub..epsilon. R.alpha..sub.774, nhFc.sub..epsilon. R.alpha..sub.1198, nhFc.sub..epsilon. R.alpha..sub.612, nhFc.sub..epsilon. R.alpha..sub.591, nhFc.sub..epsilon. R.alpha..sub.699 and/or nhFc.sub..epsilon. R.alpha..sub.516.

DETD Isolated ***Fc*** .sub..epsilon. R molecule protein of the present invention can be produced by culturing a cell capable of expressing the protein under. . . Suitable and preferred nucleic acid molecules with which to transform a cell are as disclosed herein for suitable and preferred ***Fc*** .sub..epsilon. R nucleic acid molecules per se. Particularly preferred nucleic acid molecules to include in recombinant cells of the present invention. . .

DETD . . . one nucleic acid molecule. Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing a

Fc .sub..epsilon. R molecule protein of the present invention or can be capable of producing such proteins after being transformed with at. . . .

DETD . . . transformed, examples of which are disclosed herein. A particularly preferred recombinant molecule includes pVL-nhFc.sub..epsilon. R.alpha..sub.612. Details regarding the production of ***Fc*** .sub..epsilon. R molecule nucleic acid molecule-containing recombinant molecules are disclosed herein. Particularly preferred recombinant cell of the present invention includes Trichoplusia. . . .

DETD A ***Fc*** .sub..epsilon. R molecule of the present invention can include chimeric molecules comprising a portion of a ***Fc*** .sub..epsilon. R molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the ***Fc*** .sub..epsilon. R portion binds to IgE in essentially the same manner as a ***Fc*** .sub..epsilon. R molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of. . . .

DETD A ***Fc*** .sub..epsilon. R molecule of the present invention can be contained in a formulation, herein referred to as a ***Fc*** .sub..epsilon. R formulation. For example, a ***Fc*** .sub..epsilon. R can be combined with a buffer in which the ***Fc*** .sub..epsilon. R is solubilized, and/or a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a ***Fc*** .sub..epsilon. R can function to selectively bind to IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate. . . . not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be in mixed with ***Fc*** .sub..epsilon. R or conjugated (i.e., attached) to ***Fc*** .sub..epsilon. R in such a manner as to not substantially interfere with the ability of the ***Fc*** .sub..epsilon. R to selectively bind to IgE.

DETD A ***Fc*** .sub..epsilon. R of the present invention can be bound to the surface of a cell expressing the ***Fc*** .sub..epsilon. R. A preferred ***Fc*** .sub..epsilon. R-bearing cell includes a recombinant cell expressing a nucleic acid molecule encoding a ***human*** ***Fc*** .sub..epsilon. R alpha chain of the present invention. A more preferred recombinant cell of the present invention expresses a nucleic acid. . . .

DETD In addition, a ***Fc*** .sub..epsilon. R formulation of the present invention can include not only a ***Fc*** .sub..epsilon. R but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers. . . .

DETD The present invention also includes ***human*** ***Fc*** .sub..epsilon. R mimetopes and use thereof to detect IgE. In accordance with the present invention, a "mimetope" refers to any compound that is able to mimic the ability of a ***Fc*** .sub..epsilon. R molecule to bind to IgE. A mimetope can be a peptide that has been modified to decrease its susceptibility. . . . by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of ***Fc*** .sub..epsilon. R mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex technology, peptides identified by random screening of peptide libraries and proteins. . . .

DETD One embodiment of the present invention is a method to detect non- ***human*** IgE which includes the steps of: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule with a putative IgE-containing composition under conditions suitable for formation of an ***Fc*** .sub..epsilon. R molecule:IgE complex; and (b) detecting levels of IgE by detecting said ***Fc*** .sub..epsilon. R molecule:IgE complex. Presence of such a ***Fc*** .sub..epsilon. R molecule:IgE complex indicates that the animal is producing IgE. Preferred non- ***human*** IgE to detect using a ***human*** ***Fc*** .sub..epsilon. R molecule include canine IgE, feline IgE and equine IgE. The present method can further include the step of determining whether an IgE complexed with a ***Fc*** .sub..epsilon. R molecule is heat labile. Methods to determine heat lability of IgE are disclosed in the Examples section. Preferably, an. . . . certain flea or heartworm allergens. Moreover, Applicants believe that identification of heat labile IgE and non-heat labile IgE using a ***human*** ***Fc*** .sub..epsilon. R

suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE. As such, a ***Fc*** .sub..epsilon. R molecule of the present invention may be useful for detecting molecules bound by the ***Fc*** .sub..epsilon. R molecule but not identical to a known IgE.

DETD As used herein, the term "contacting" refers to combining or mixing, in this case a putative IgE-containing composition with a ***human*** ***Fc*** .sub..epsilon. R molecule. Formation of a complex between a ***Fc*** .sub..epsilon. R and an IgE refers to the ability of the ***Fc*** .sub..epsilon. R to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a ***Fc*** .sub..epsilon. R of the present invention to preferentially bind to IgE, without being able to substantially bind to other antibody isotypes. Binding between a ***Fc*** .sub..epsilon. R and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures,. . .

DETD . . . complexes are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between ***Fc*** .sub..epsilon. R and any IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in. . .

DETD . . . visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a ***detectable*** ***marker*** . In other assays, conjugation (i.e., attachment) of a ***detectable*** ***marker*** to the ***Fc*** .sub..epsilon. R or to a reagent that selectively binds to the ***Fc*** .sub..epsilon. R or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable. . . biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure.RTM. NeutrAvidin). Preferably, biotin is conjugated to an alpha chain of a ***Fc*** .sub..epsilon. R. Preferably a carbohydrate group of the ***Fc*** .sub..epsilon. R alpha chain is conjugated to biotin. A preferred ***Fc*** .sub..epsilon. R molecule conjugated to biotin comprises PhFc.sub..epsilon. R.alpha..sub.172 -BIOT (the production of which is described in the Examples section).

DETD In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a ***Fc*** .sub..epsilon. R molecule that is conjugated to a ***detectable*** ***marker*** . A suitable ***detectable*** ***marker*** to conjugate to a ***Fc*** .sub..epsilon. R molecule includes, but is not limited to, a radioactive label, a fluorescent label, a chemiluminescent label or a chromophoric label. A ***detectable*** ***marker*** is conjugated to a ***Fc*** .sub..epsilon. R molecule or a reagent in such a manner as not to block the ability of the ***Fc*** .sub..epsilon. R or reagent to bind to the IgE being detected. Preferably, a carbohydrate group of a ***Fc*** .sub..epsilon. R is conjugated to biotin.

DETD In another embodiment, a ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting a putative IgE-containing composition with a ***Fc*** .sub..epsilon. R molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the ***Fc*** .sub..epsilon. R molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, a ***Fc*** .sub..epsilon. R molecule, an antigen, an antibody and a lectin, depending upon which portion of the ***Fc*** .sub..epsilon. R molecule:IgE complex being detected. Preferred identifying labeled compounds that are antibodies include, for example, anti-IgE antibodies and anti- ***Fc*** .sub..epsilon. R antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a ***Fc*** .sub..epsilon. R molecule of the present invention produced in insect cells. An indicator molecule itself can be attached to a ***detectable*** ***marker*** of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

DETD In one preferred embodiment, a ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to a ***Fc*** .sub..epsilon. R molecule of the

present invention. Examples of such a reagent includes, but are not limited to, an antibody that selectively binds to a ***Fc*** .sub..epsilon. R molecule (referred to herein as an anti- ***Fc*** .sub..epsilon. R antibody) or a compound that selectively binds to a ***detectable*** ***marker*** conjugated to a ***Fc*** .sub..epsilon. R molecule. ***Fc*** .sub..epsilon. R molecules conjugated to biotin are preferably detected using streptavidin, more preferably using ImmunoPure.RTM. NeutrAvidin (available from Pierce, Rockford, Ill.).

DETD In another preferred embodiment, a ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to an IgE antibody (referred. . . cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., an ***Fc*** receptor), and an antibody-binding complement protein. Preferred anti-IgE reagents include, but are not limited to, D9, and CMI antibody #9,. . .

DETD . . . beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a ***detectable*** ***marker*** .

DETD A preferred immunoabsorbent assay method includes a step of either: (a) binding an ***Fc*** .sub..epsilon. R molecule to a substrate prior to contacting a ***Fc*** .sub..epsilon. R molecule with a putative IgE-containing composition to form a ***Fc*** .sub..epsilon. R molecule-coated substrate; or (b) binding a putative IgE-containing composition to a substrate prior to contacting a ***Fc*** .sub..epsilon. R molecule with a putative IgE-containing composition to form a putative IgE-containing composition-coated substrate. Preferably, the substrate includes of a non-coated substrate, a ***Fc*** .sub..epsilon. R molecule-coated substrate, an antigen-coated substrate or an anti-IgE antibody-coated substrate.

DETD . . . upon whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, a ***Fc*** .sub..epsilon. R molecule of the present invention is used as a capture molecule when the ***Fc*** .sub..epsilon. R molecule is bound to a substrate. Alternatively, a ***Fc*** .sub..epsilon. R molecule is used as an indicator molecule when the ***Fc*** .sub..epsilon. R molecule is not bound to a substrate. Suitable molecule for use as capture molecules or indicator molecules include, but are not limited to, a ***Fc*** .sub..epsilon. R molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

DETD . . . binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a ***detectable*** ***marker***) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a ***detectable*** ***marker***) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules. . .

DETD . . . molecule that can selectively bind to an IgE bound to the antigen, the indicator molecule can be conjugated to a ***detectable*** ***marker*** (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . . and the substrate is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is a ***Fc*** .sub..epsilon. R molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

DETD In one embodiment, a ***Fc*** .sub..epsilon. R molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well. . . A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for ***Fc*** .sub..epsilon. R molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain ***Fc*** .sub..epsilon. R molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the ***Fc*** .sub..epsilon. R is added to the substrate and incubated to allow formation of a complex between the indicator molecule and the ***Fc*** .sub..epsilon. R molecule:IgE complex. Preferably, the indicator molecule is conjugated to a ***detectable***

marker (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand. . .

DETD . . . material, if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE complex binding to the substrate. A ***Fc*** .sub..epsilon. R molecule is added to the substrate and incubated to allow formation of a complex between the ***Fc*** .sub..epsilon. R molecule and the anti-IgE antibody:IgE complex. Preferably, the ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess ***Fc*** .sub..epsilon. R molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device. . .

DETD . . . Excess non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A ***Fc*** .sub..epsilon. R molecule is added to the substrate and incubated to allow formation of a complex between the ***Fc*** .sub..epsilon. R molecule and the IgE. Preferably, the ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** (preferably to biotin, an enzyme label or a fluorescent label). Excess ***Fc*** .sub..epsilon. R molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device. . .

DETD . . . or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a ***detectable*** ***marker*** , preferably a calorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample. . . zone which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case a ***Fc*** .sub..epsilon. R molecule, as disclosed above, that immobilizes the IgE complexed to the antigen in the capture zone. The capture reagent. . .

DETD . . . apparatus used to detect IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a ***Fc*** .sub..epsilon. R molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a. . .

DETD . . . assay in which the presence of IgE in a putative IgE-containing composition is determined by adding such composition to a ***Fc*** .sub..epsilon. R molecule of the present invention and an isolated IgE known to bind to the ***Fc*** .sub..epsilon. R molecule. The absence of binding of the ***Fc*** .sub..epsilon. R molecule to the known IgE indicating the presence of IgE in the putative IgE-containing composition.

DETD . . . detect IgE based on each of the disclosed detection methods. One embodiment is a kit to detect IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule and a means for detecting an IgE including canine IgE, feline IgE and/or equine IgE. Suitable and preferred ***Fc*** .sub..epsilon. R molecules are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the ***Fc*** .sub..epsilon. R molecule or to an IgE. A preferred kit of the present invention further comprises a detection means including one. . . herein, an antibody capable of selectively binding to an IgE disclosed herein and/or a compound capable of binding to a ***detectable*** ***marker*** conjugated to a ***Fc*** .sub..epsilon. R molecule (e.g., avidin, streptavidin and ImmunoPure.RTM. NeutrAvidin when the ***detectable*** ***marker*** is biotin). Such antigens preferably induce IgE antibody production in animals including canines, felines and/or equines.

DETD . . . present invention is a general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon. R molecule of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that. . .

DETD . . . such as cod, halibut or and tuna, egg, milk, Brewer's yeast, whole wheat, corn, soybean, cheese and rice, and a ***human*** ***Fc*** .sub..epsilon. R molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

DETD This example describes the construction of a recombinant baculovirus expressing a truncated portion of the .alpha.-chain of the ***human*** ***Fc*** .sub..epsilon. receptor.

DETD Recombinant molecule pVL-nhFc.sub..epsilon. R.alpha..sub.612, containing a nucleic acid molecule encoding the extracellular domain of the ***Fc*** .sub..epsilon. R .alpha. chain, operatively linked to baculovirus polyhedron transcription control sequences was produced in the following manner. A cDNA clone encoding the full-length alpha chain (.alpha. chain) of the ***human*** ***Fc*** .sub..epsilon. receptor was obtained from Dr. Jean-Pierre Kinet (Harvard University, Cambridge, Mass.). The cDNA clone included an about 1198 nucleotide insert,. . . SEQ ID NO: 1. Translation of SEQ ID NO: 1 indicates that nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.1198 encodes a full-length ***human*** ***Fc*** .sub..epsilon. receptor .alpha. chain protein of about 257 amino acids, referred to herein as PhFc.sub..epsilon. R.alpha..sub.257, having amino acid sequence SEQ. . . 1. The complement of SEQ ID NO: 1 is represented herein by SEQ ID NO:3. The proposed mature protein (i.e., ***Fc*** .sub..epsilon. R.alpha. chain from which the signal sequence has been cleaved), denoted herein as PhFc.sub..epsilon. R.alpha..sub.232, contains about 232 amino acids. . . .

DETD To produce a secreted form of the extracellular domain of the ***Fc*** .sub..epsilon. R .alpha. chain, the hydrophobic transmembrane domain and the cytoplasmic tail of the ***Fc*** .sub..epsilon. R .alpha. chain encoded by nhFc.sub..epsilon. R.alpha..sub.1198 were removed as follows. A ***Fc*** .sub..epsilon. R .alpha. chain extracellular domain nucleic acid molecule-containing fragment of about 612 nucleotides was PCR amplified from nhFc.sub..epsilon. R.alpha..sub.1198 using. . . produce nhFc.sub..epsilon. R.alpha..sub.612. Nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.612 contained an about 591 nucleotide fragment encoding the extracellular domain of the ***human*** ***Fc*** .sub..epsilon. R .alpha. chain, extending from about nucleotide 107 to about nucleotide 697 of SEQ ID NO 1, denoted herein as. . . denoted SEQ ID NO: 10. Translation of SEQ ID NO: 10 indicates that nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.612 encodes a ***Fc*** .sub..epsilon. R protein of about 197 amino acids, referred to herein as PhFc.sub..epsilon. R.alpha..sub.197, having amino acid sequence SEQ ID NO: 11. Nucleic acid molecule nhFc.sub..epsilon. R.alpha..sub.612 encodes a secretable form of the ***human*** ***Fc*** .sub..epsilon. R .alpha. chain which does not possess a leader sequence, which is denoted herein as PhFc.sub..epsilon. R.alpha..sub.172 having amino acid. . .

DETD This example describes the biotinylation of a recombinant ***human*** ***Fc*** .sub..epsilon. R alpha chain protein.

DETD The results shown in FIG. 1 indicate that the alpha chain of ***human*** ***FcR*** (solid squares) detects the presence of canine IgE in a solid-phase assay in a similar manner as the control antibody. . . .

DETD The results shown in FIG. 2 indicate that the alpha chain of ***human*** ***Fc*** .sub..epsilon. R detects the presence of canine IgE antibodies that bind specifically to a common grass allergen or to a common. . .

DETD . . . in FIG. 3 indicate that canine IgE from a variety of dog sera are detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R in a manner similar to using an antibody that binds specifically to canine IgE. The absence of detectable amounts. . .

DETD . . . 4 indicate that canine IgE that binds specifically to a flea saliva antigen is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R.

DETD . . . IgE from dogs allergic to flea saliva and from dogs infected with heartworm are detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of calorimetric signal in samples of heat inactivated sera indicates that antibody bound to the anti-IgE antibody and detected by ***Fc*** .sub..epsilon. R alpha chain is an epsilon isotype antibody and not another isotype.

DETD . . . indicate that canine IgE that binds specifically to flea saliva, contained in serum, is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of colorimetric signal in samples of heat inactivated serum indicates that antibody bound to the flea saliva protein and detected by ***Fc*** .sub..epsilon. R alpha chain is an epsilon isotype antibody.

DETD . . . feline IgE that binds specifically to crude homogenate of heartworm or Di33 protein is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R.

DETD . . . feline IgE from heartworm-infected cats that specifically binds to the heartworm antigen Di33 is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of calorimetric signal in samples of heat inactivated sera indicates that antibody bound to the Di33 protein and detected by ***Fc*** .sub..epsilon. R alpha chain is an epsilon isotype antibody.

DETD . . . be allergic to certain allergens specifically binds to certain plant and mite allergens is detected using the alpha chain of ***human*** ***Fc*** .sub..epsilon. R.

DETD This example describes detection of canine IgE in a solid-phase ELISA using basophilic cells transfected with ***human*** ***Fc*** .sub..epsilon. R alpha chain.

DETD Rat basophilic leukemia (RBL) cells transfected with a nucleic acid molecule encoding a ***human*** ***Fc*** .sub..epsilon. R alpha chain (referred to herein as RBL-hFc.sub..epsilon. R cells; described in Miller et al., Science 244:334-337, 1989) were used. . . .

DETD The results shown in FIG. 10 indicate that canine IgE from heartworm-infected dogs (.diamond-solid.) is detected using RBL-h ***Fc*** .sub..epsilon. R cells expressing the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of calorimetric signal in samples of heat inactivated samples of such sera (.box-solid.) indicates that antibody detected by the ***Fc*** .sub..epsilon. R alpha chain on the RBL-h ***Fc*** .sub..epsilon. R cells is an epsilon isotype antibody. Similarly, the results shown in FIG. 11 indicate that canine IgE from dogs sensitized with flea saliva (.diamond-solid.) is detected using RBL-h ***Fc*** .sub..epsilon. R cells expressing the alpha chain of ***human*** ***Fc*** .sub..epsilon. R. In addition, the absence of colorimetric signal in samples of heat inactivated samples of such sera (.box-solid.) indicates that antibody detected by the ***Fc*** .sub..epsilon. R alpha chain on the RBL-h ***Fc*** .sub..epsilon. R cells is an epsilon isotype antibody.

CLM What is claimed is:

1. A method to detect IgE comprising: (a) contacting an isolated ***human*** ***Fc*** .sub..epsilon. receptor (***Fc*** .sub..epsilon. R) molecule comprising at least a portion of a ***human*** ***Fc*** .sub..epsilon. R alpha chain that binds to IgE with a putative IgE-containing composition under conditions suitable for formation of a ***Fc*** .sub..epsilon. R molecule:IgE complex, wherein said IgE is selected from the group consisting of canine IgE, feline IgE and equine IgE; and (b) determining the presence of IgE by detecting said ***Fc*** .sub..epsilon. R molecule:IgE complex, the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex indicating the presence of IgE.

2. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule comprises a protein selected from the group consisting of PhFc.sub..epsilon. R.alpha..sub.257, PhFc.sub..epsilon. R.alpha..sub.197, PhFc.sub..epsilon. R.alpha..sub.232 and PhFc.sub..epsilon. R.alpha..sub.172.

. . .

3. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of nhFc.sub..epsilon. R.alpha..sub.774, nhFc.sub..epsilon. R.alpha..sub.1198, nhFc.sub..epsilon.. . . .

4. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising. . .

5. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** .

6. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a. . .

7. The method of claim 1, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, biotin-related compounds, avidin, avidin-related compounds and a peroxidase.

. . .

8. The method of claim 1, wherein a carbohydrate group of said ***Fc*** .sub..epsilon. R molecule is conjugated to biotin.

13. The method of claim 1 further comprising the step selected from the group consisting of binding said ***Fc*** .sub..epsilon. R molecule to a substrate prior to performing step (a) to form a ***Fc*** .sub..epsilon. R molecule-coated substrate and binding said putative IgE-containing composition to a substrate prior to performing step (a) to form a putative IgE-containing composition-coated substrate, wherein said substrate is selected from the group consisting of a non-coated substrate, a ***Fc*** .sub..epsilon. R molecule-coated substrate, an antigen-coated substrate and an anti-IgE antibody-coated substrate.

21. The method of claim 1, wherein said step of detecting comprises: (a) contacting said ***Fc*** .sub..epsilon. R molecule:IgE complex with an indicator molecule that binds selectively to said ***Fc*** .sub..epsilon. R molecule:IgE complex; (b) removing substantially all of said indicator molecule that does not selectively bind to ***Fc*** .sub..epsilon. R molecule:IgE complex; and (c) detecting said indicator molecule, wherein presence of said indicator molecule is indicative of the presence. . . .

. . . . 22. The method of claim 21, wherein said indicator molecule comprises a compound selected from the group consisting of a ***Fc*** .sub..epsilon. R molecule, an antigen, an antibody and a lectin.

23. The method of claim 1, said method comprising the steps of: (a) immobilizing said ***Fc*** .sub..epsilon. R molecule on a substrate; (b) contacting said ***Fc*** .sub..epsilon. R molecule with said putative IgE-containing composition under conditions suitable for formation of an ***Fc*** .sub..epsilon. R molecule:IgE complex bound to said substrate; (c) removing non-bound material from said substrate under conditions that retain ***Fc*** .sub..epsilon. R molecule:IgE complex binding to said substrate; and (d) detecting the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex.

24. The method of claim 23, wherein the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting said ***Fc*** .sub..epsilon. R molecule:IgE complex with a compound selected from the group consisting of an antigen and an antibody that binds selectively. . . .

25. The method of claim 24, wherein said compound comprises a ***detectable*** ***marker*** .

26. The method of claim 1, said method comprising the steps of: (a) immobilizing a desired antigen on a substrate;. . . . binding to said substrate; and (d) detecting the presence of said antigen:IgE complex by contacting said antigen:IgE complex with said ***Fc*** .sub..epsilon. R molecule.

27. The method of claim 26, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, avidin, a peroxidase and other members of the. . .

. . . . binding to said substrate; and (d) detecting the presence of said antibody:IgE complex by contacting said antibody:IgE complex with said ***Fc*** .sub..epsilon. R molecule.

29. The method of claim 28, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, a biotin-related compound, avidin, an avidin-related compound and. . .

. . . . method comprising the steps of: (a) immobilizing said putative IgE-containing composition on a substrate; (b) contacting said composition with said ***Fc*** .sub..epsilon. R molecule under conditions suitable for formation of an ***Fc*** .sub..epsilon. R molecule:IgE complex bound to said substrate; (c) removing non-bound material from said substrate under conditions that retain ***Fc*** .sub..epsilon. R molecule:IgE complex binding to said substrate; and (d) detecting the presence of said ***Fc*** .sub..epsilon. R molecule:IgE

complex.

31. The method of claim 30, wherein the presence of said ***Fc*** .sub..epsilon. R molecule:IgE complex is detected by contacting said ***Fc*** .sub..epsilon. R molecule:IgE complex with an indicator molecule selected from the group consisting of an antibody, an antigen and a lectin.

. . .

32. The method of claim 30, wherein said ***Fc*** .sub..epsilon. R molecule comprises a ***detectable*** ***marker*** .

35. A kit for detecting IgE comprising a ***human*** ***Fc*** .sub..epsilon. receptor molecule comprising at least a portion of a ***human*** ***Fc*** .sub..epsilon. R alpha chain that binds to IgE and a means for detecting an IgE selected from the group consisting of.

. .

38. The kit of claim 35, wherein said detection means detects said ***Fc*** .sub..epsilon. R molecule.

39. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to biotin.

46. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule comprises a protein selected from the group consisting of PhFc.sub..epsilon. R.alpha..sub.257, PhFc.sub..epsilon. R.alpha..sub.197, PhFc.sub..epsilon. R.alpha..sub.232 and PhFc.sub..epsilon. R.alpha..sub.172.

. . .

47. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of nhFc.sub..epsilon. R.alpha..sub.774, nhFc.sub..epsilon. R.alpha..sub.1198, nhFc.sub..epsilon.. . .

48. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising. . .

49. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** .

50. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a. . .

51. The kit of claim 35, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, a biotin-related compound, avidin, an avidin-related compound and. . .

52. The kit of claim 35, wherein a carbohydrate group of said ***Fc*** .sub..epsilon. R molecule is conjugated to biotin.

. . . said labeling reagent is impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising said ***Fc*** .sub..epsilon. R molecule, wherein said capture reagent is located downstream of said labeling reagent within a capture zone fluidly connected to. . .

65. A general allergen kit comprising an allergen common to all regions of the United States and a ***human*** ***Fc*** .sub..epsilon. receptor molecule comprising at least a portion of a ***human*** ***Fc*** .sub..epsilon. R alpha chain that binds to IgE.

71. The kit of claim 65, wherein said ***Fc*** .sub..epsilon. R molecule comprises a protein selected from the group consisting of PhFc.sub..epsilon. R.alpha..sub.257, PhFc.sub..epsilon. R.alpha..sub.197, PhFc.sub..epsilon. R.alpha..sub.232 and PhFc.sub..epsilon. R.alpha..sub.172.

. . .

72. The kit of claim 65, wherein said ***Fc*** .sub..epsilon. R molecule is encoded by a nucleic acid molecule selected from the group consisting of nhFc.sub..epsilon. R.alpha..sub.774, nhFc.sub..epsilon. R.alpha..sub.1198, nhFc.sub..epsilon.. . .

73. The kit of claim 65, wherein said ***Fc*** .sub..epsilon. R

molecule is encoded by a nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising. . .

74. The kit of claim 65, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** .

75. The kit of claim 65, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a ligand.
. . .

76. The kit of claim 65, wherein said ***Fc*** .sub..epsilon. R molecule is conjugated to a ***detectable*** ***marker*** selected from the group consisting of fluorescein, a radioisotope, a phosphatase, biotin, a biotin-related compound, avidin, an avidin-related compound and. . .

77. The kit of claim 65, wherein a carbohydrate group of said ***Fc*** .sub..epsilon. R molecule is conjugated to biotin.

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